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Observations on the influence of food supply and temperature on the feeding and growth of the larvae of *Ostrea edulis* L.

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Observations on the influence of food supply and temperature on the feeding and growth of the larvae of *Ostrea edulis* L.

INTRODUCTION

THIS paper is concerned with the effect of variations in the abundance of food and temperature on the feeding and growth of the larvae of *O. edulis*. Many of the earlier laboratory studies (Cole, 1937; Bruce, Knight & Parke, 1940; Walne, 1956a) have been almost exclusively concerned with finding suitable food organisms, and the techniques of laboratory culture have been arrived at by a process of trial and error. Present methods now make it possible to culture larvae on a sufficiently reliable basis to provide a good supply of larvae of all sizes, and detailed investigations into the effect on the larvae of a wide variety of conditions have become practicable. From such experiments, improvements in the method of culture have been achieved and some light is thrown on the reaction of the larvae to various field conditions.

MATERIAL AND METHODS

WHEN testing the influence of some factor on the larvae, it is possible to make two rather dissimilar types of experiment. Either freshly liberated larvae can be placed in some particular experimental condition and the experiment continued until all the larvae are either dead or have metamorphosed; or, alternatively, the larvae can be subjected to the experimental condition for a short period, one of their qualities being taken as an index of their reaction to the condition. That is, the reaction of the larvae can be either chronically measured or acutely measured (Bullock, 1955). The first method is in many ways the best since the proportion of the larvae which metamorphose and the time taken are of the first importance. The major difficulty is that of maintaining the experimental conditions for a period of up to 3–4 weeks, and of controlling the various changes that will be continuously in progress in the culture throughout the period; changes which come about when seawater is confined in small vessels, the accumulation of dead larvae, faeces and excretory products, and possibly variations in the quality of the food supply, all of which may overshadow the effect of the factor which it is desired to measure. In practice these long experiments are very time-consuming, and it becomes difficult to make replicate experiments with other broods of larvae.

In acutely measured experiments, lasting for 24 to 48 hours, less relative change occurs in the environment, and there is little change in larval density due to mortality. The reaction of the larvae can be gauged by observations on their growth and feeding. This method has given valuable results and has permitted the same factor to be tested on larvae of different sizes and origins with a reasonable economy of time. It does not, however, allow of the possibility of the larvae becoming acclimatized to the experimental conditions.

A description is given first of the general methods used during this work, followed by a detailed description of the special methods used to gauge the reaction of the larvae to their environment.

GENERAL METHODS

Larvae were obtained by culturing 12–15 parent oysters in 40 litre tanks through which seawater, warmed to 20–22°C and enriched with *Phaeodactylum tricornutum* Bohlin, flowed continuously. By this treatment larvae were available from early February to September. When larvae had been liberated into the tank, they were removed by filtering the water through either a stainless steel or a silk screen and then cultured in standard conditions (Walne, 1959). Samples for experimental purposes were obtained, as required, from these standard cultures. The method of maintaining the standard culture is briefly described in the following paragraph.

After washing several times with filtered seawater, the larvae were suspended in seawater in a graduated cylinder and after obtaining a homogeneous suspension by stirring with a perforated plastic plunger, samples of either 1·0 ml or 0·5 ml were withdrawn with an automatic pipette. The number of larvae in the samples was then estimated by counting on a squared plate under a binocular microscope and their sizes by measuring the maximum length of the shells under a microscope, using a 2/3 in. objective and a $\times 10$ eyepiece. The micrometer eyepieces and draw tube extension used were such that a single division represented either 5μ or 10μ, and the larvae were measured to the nearest division below. Sufficient of the larval suspension was then added to the glass or fibreglass culture vessel to give a density of 5,000–10,000 per litre. Sufficient culture of *Isochrysis galbana* Parke was added to give a cell density of 75–100 cells/μl and, in those experiments with high densities of larvae, sufficient culture was added every 3 hours to maintain this level. The vessels stood in a controlled temperature room which maintained the water temperature at about 21°C. The water was changed every 48 hours; the larvae were collected by siphoning through a sieve, then washed and replaced in clean water. In the majority of cultures antibiotics were added to control the growth of bacteria (Walne, 1958a).

The density of algal cells was determined by counting samples in a haemacytometer. The densities used are reported as 'cells per microlitre' ($1\mu l = 1\ mm^3 = 10^{-3}$ ml), a term which is preferable to 'mm^3' as it is more obviously comparable with the 'ml' and the 'litre' used for the larger and less dense phytoplankton. In this paper the term 'density of cells' refers to the number of cells per unit volume of water and carries no implication as to their specific gravity. An index of the size of cells of the various species used was obtained by centrifuging cultures of known cell density in graduated haematocrit tubes and observing the percentage of the total volume of culture which was occupied by packed cells. It was usually necessary first to concentrate the culture in normal centrifuge tubes and then resuspend the cells in a known volume of medium in a graduated micro-flask.

Seawater was obtained from the estuary of the river Conway and usually had a salinity of 31–32‰. Prior to use it was passed through a sand or ceramic filter, which removed practically all the particulate material, and then stored for a period under an ultra-violet strip light which reduced the bacterial flora to a very low level. In all experiments reported in this paper 50 i.u. of penicillin G and 0·25 mg of streptomycin sulphate were added per ml to inhibit the growth of bacteria (Walne, 1958a).

The experiments were made in hard glass beakers or flasks holding 100 to 1,000 ml of seawater. Gentle stirring was provided by a stream of air bubbles from a glass jet held near the bottom of the vessel, and temperature control by standing the vessels in thermostatically controlled water baths, or in a temperature controlled room. The lighting was subdued daylight, resulting in a low algal division rate.

EXPERIMENTS USING ^{32}P

If a solution of the radioactive isotope of phosphorus, ^{32}P, is added to the medium in which nannoplankton organisms are being cultured, the phosphorus is taken up and incorporated into the cells. The cells are then said to be 'marked' and their fate and the subsequent movement of phosphorus can be followed. Marshall and Orr (1955a, b) fed marked cells

of various species of algae to the copepod *Calanus finmarchicus* and by assaying the *Calanus* for radioactivity at the end of the experiment the minimum number of cells which the animal must have eaten during the experimental period could be calculated. By collecting and assaying the faecal pellets as well, it was possible to calculate the total number of cells eaten by the animal, and the proportion assimilated into the tissues and the proportion egested. Marked cells have also been used to study the pumping and filtering efficiency of *Pecten irradians* (Chipman and Hopkins, 1954) and *Mercenaria mercenaria* (Smith, 1958).

Radioactive cultures of *Isochrysis galbana* and other photosynthetic nannoplankton organisms were prepared by adding a dense inoculum to 100 ml of sterile seawater enriched with 0·2 g Na NO_3/litre and about 0·1 mc of ^{32}P. The inoculum was prepared by centrifuging an actively growing culture and resuspending the cells in a little medium. In this way little of the original medium was carried over and an initial cell density of 10,000–20,000 cells/μl was obtained in the culture. As the ^{32}P was obtained in the form of carrier-free orthophosphate, its addition to the medium was chemically negligible, and the only non-radioactive phosphorus present was that in the seawater or carried over from the original medium. This deficiency of phosphorus with respect to nitrate, and the high cell density, resulted in most of it being rapidly taken up into the cells. For example, in a culture of *I. galbana* 48 hours old, the cell density was 22,000 per μl and less than 1% of the ^{32}P remained in the medium. A high degree of uptake by the cells was important as this ensured that the larvae could only obtain ^{32}P by eating and digesting the cells. As centrifuging the culture before use resulted in the cells clumping together and not dispersing for 24 hours, it was not possible to remove the medium by this method. The cultures were continuously illuminated with a fluorescent light.

It has been shown (Rice, 1953) that in *Nitzschia* cultured in the presence of an abundance of phosphorus there is a considerable amount which, although taken up by the cells, is very labile, and will readily leave the cells if they are placed in water with a low phosphate content. It is presumably in solution in the cell sap. As the medium becomes exhausted this labile phosphorus is gradually used up and converted into the organic fraction and this is not readily lost by the cells. The *Isochrysis* cultures followed a similar pattern and, if they were used 2–3 days after sub-culturing, little exchange took place between the cells and the seawater when they were fed to the larvae. In experiments where it was desired to calculate the number of cells assimilated by the larvae, it was necessary to estimate the amount of ^{32}P in the cells at the beginning and end of the experiment. The change over 24 hours did not normally exceed 2–3%.

Because of the rapid exhaustion of phosphorus in the very dense cultures the cells soon became immobile. This could be avoided by adding about 25% by volume of 'Erdschreiber' medium once phosphorus exhaustion had occurred, and in this way vigorous cultures of motile, radioactive cells, with little ^{32}P in the medium, could be maintained for 10 days or more.

In the feeding experiments there was some loss of ^{32}P from the water. Experiments showed that this was due to the growth of bacteria on the sides of the flasks and to some extent to the settlement and attachment of the *Isochrysis* or other algal cells to the flask. In one experiment with filtered seawater and inorganic ^{32}P but no algae or larvae, 18% disappeared from the medium in 24 hours. In a duplicate experiment, with penicillin and streptomycin added, the loss was reduced to 6%, and in a third experiment, which was heat sterilised, no phosphorus was lost. At the usual experimental density of about 1 larva per 5 ml the larvae themselves did not remove more than 1% of the ^{32}P.

Samples for radioactive assay were placed on shallow aluminium trays, 25 mm in diameter, and dried on a hot plate. To avoid crystals of salt accidentally contaminating the counter, the dried samples of both larvae and medium were sealed to the trays with a few drops of 1% celloidon. The samples of medium (usually 0·4 ml) were measured with semi-automatic micropipettes and dispersed evenly over the trays by adding a drop of 'Teepol'. To determine the relative proportions of ^{32}P in the water and in the cells a few ml of culture were filtered through a double thickness of Whatman's No. 42 filter paper. This treatment satisfactorily removed the algal cells without giving any appreciable absorption of the ^{32}P from the medium.

Self-absorption of radioactivity by the sample has not been important in this work partly because it was fairly low (about 15% for a 0·4 ml seawater sample), but mainly because of the standard nature of the sample used. The samples of water and of larvae contained roughly equal amounts of salts, and self-absorption would therefore be the same and the proportions (rather than the absolute terms) with which this work has been concerned would be unchanged.

The counting equipment comprised a Geiger-Müller tube, shielded by a lead castle, with an aluminium end window (7 mg/cm²) connected through a probe unit to a scaling circuit. After the appropriate corrections for background, decay and resolving time (Taylor, 1951, Francis et al., 1954) the assay of each sample was expressed as 'counts per minute' (c.p.m.).

Larvae were prepared for assay by emptying the culture into a beaker and adding a few drops of formalin. The culture was then swirled round, and the larvae settled to the bottom and collected into the centre where they were easily pipetted into a dish of clean seawater. A single washing of the larvae was found to reduce the amount of radioactivity carried over from the experimental medium to negligible proportions. After washing they were pipetted onto the aluminium counting trays, those of each experiment usually being divided between two or three trays, and the number on each tray counted under a binocular microscope. The larvae were roughly distributed over the tray and not allowed to congregate round the edge. This gave satisfactory results, although the geometry of the presentation of the larvae to the counter was not as uniform as if the larvae had been dissolved and uniformly distributed over the sample tray.

The radioactivity associated with the live larvae was not readily removed and only a few percent was lost overnight if they were fed on unmarked *Isochrysis*. With killed larvae preserved in formalin 25–50% of the radioactivity is lost overnight and it was therefore essential to transfer the larvae to sample trays as soon as they were killed. Marshall & Orr (1961) have found that ^{32}P is liberated very rapidly from killed *Calanus*.

The larvae could take up ^{32}P from the inorganic fraction in the medium, but the amount was very small compared with that assimilated with the food. The results (Fig. 1) of several series of experiments in which larvae were kept for about 24 hours in seawater enriched with ^{32}P show that the uptake was quite small, and in normal experiments, where most of the isotope was in the cells, the inorganic fraction in the water would account for only ½–1% of the total uptake.

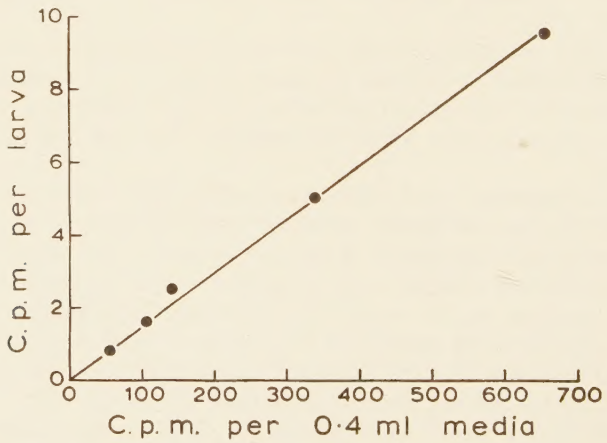

Fig. 1. *The uptake of inorganic ^{32}P by larvae cultured in various concentrations for* 48 *hours.*

GROWTH

Larvae grow 10–20 microns in length in 24 hours, an increase of 3–10%, and, providing sufficiently large samples are measured, an accurate measure of the growth can be obtained and this is a valuable check on the validity of the radioactive technique. Growth is much

easier to measure in young larvae than when they are older because when first liberated the spread of sizes is only 20 or 30 microns, with the size groups approximately normally distributed, but as the brood approaches metamorphosis the spread increases to 80 or 90 microns, and it is necessary to measure 200 or more larvae from each experiment in order to arrive at a satisfactory estimate of the mean. It is possible to grade the larvae to some extent by using stainless steel test sieves of suitable mesh, but this did not give adequate separation.

An attempt was made to measure the growth of the larvae in some radioactive experiments, but this was found to be impracticable because of the necessity to put the larvae on the assay trays within a few hours of their removal from the experiment. This gave little time for their measurement and it became the practice to run duplicate experiments with non-radioactive *Isochrysis* and to preserve these larvae for subsequent measuring.

THE SIZE AND SHAPE OF OYSTER LARVAE

The size of bivalve larvae is generally expressed by the maximum length of the shell but for some purposes purely linear dimensions are unsatisfactory. A number of determinations have therefore been made of the inter-relationship of shell length, the dry weight, and the volume when the shells are closed. In addition observations were made on the rate of sinking of larvae.

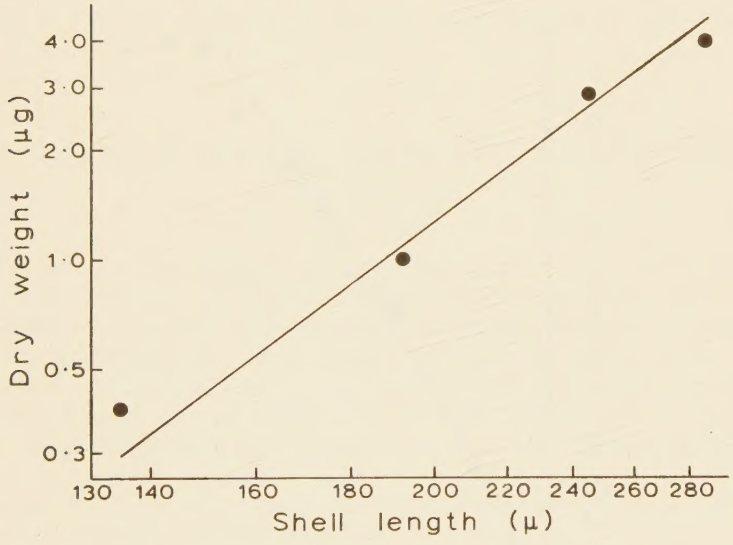

Fig. 2. *The relation between the mean dry weight of whole larvae and the shell length (on logarithmic scales).*

The dry weight (Fig. 2) was determined by pipetting random samples of a population of larvae onto a squared slide, counting them, and then washing into a tared bottle where they were repeatedly washed with distilled water to remove salt. They were weighed after drying to constant weight at 100°C. The relation between length and dry weight is linear when plotted on a log/log scale. Larvae have a calcareous shell which is a significant part of the dry weight and the percentage which it constitutes of the total weight has been determined. Three samples of larvae were dried at 100°C on a fritted glass crucible and, after weighing,

were washed with dilute HCl until effervescence stopped and again dried and weighed. The results listed below indicate that about 25% of the dry weight is organic matter.

Size of larvae	*Wt. of dry sample*	*% loss of dry weight on washing with HCl*
163 μ	0·448 g	74·6
175 μ	0·592 g	79·7
257 μ	0·012 g	68·0

The larval volume (Fig. 3) was determined by measuring the volume of scale models made in 'Plasticine'. The plan of the side view of the larvae was obtained by projecting the image obtained with a micro-projector onto squared paper and the thickness by measuring a number of suitably orientated larvae under the microscope. The relation between length and volume is approximately linear when plotted on a log/log scale. The volume increases by about 5 times during the planktonic phase, and as this is about the same factor as the increase in dry weight it indicates that the specific gravity of the larvae is roughly constant during the planktonic phase.

Fig. 3. *The relation between volume and shell length of larvae* (*on logarithmic scales*).

To obtain an indication of the work which has to be done by larvae when keeping their position in the water, the sinking rate (Fig. 4) of a number of closed larvae was determined. This roughly doubles (from 18 cm/min to 36 cm/min) during the planktonic phase.

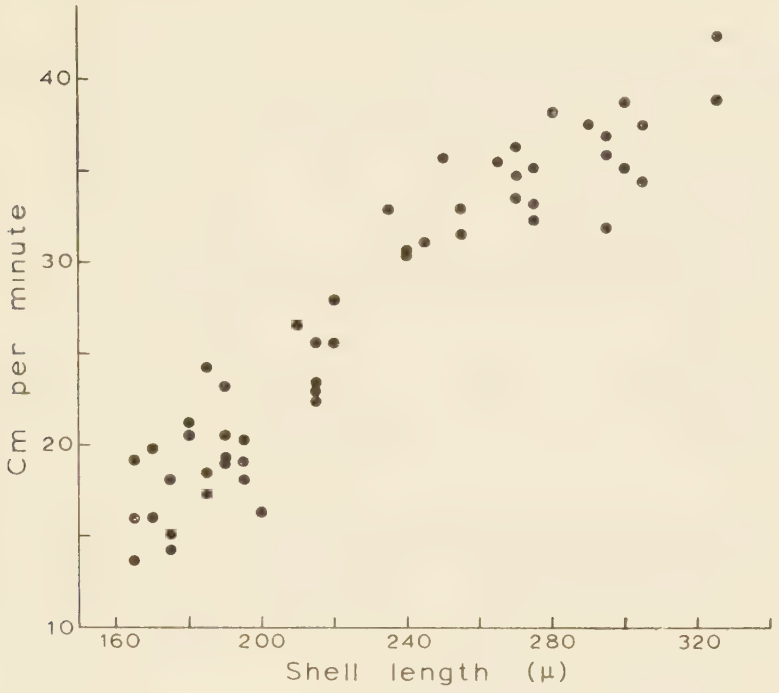

Fig. 4. *The rate of sinking of closed larvae related to the shell length.*

THE CONCENTRATION OF FOOD IN THE WATER

THE food value of a number of algal species to bivalve larvae has received a good deal of attention (see Bruce, Knight and Parke, 1940, Walne, 1956a, Davis and Guillard, 1958), but the importance of variations in the abundance of suitable foods in the water has received little notice. The writer (Walne, 1956a) using *I. galbana* obtained a general trend towards more rapid growth and spatfall when larvae were cultured with increasing densities of food in the range 10–120 cells per μl. Davis and Guillard (1958) reported experiments with *Isochrysis* and *Monochrysis* in which increased growth rates were obtained at the higher feeding rates when between 25 and 400 cells were added per μl of culture daily, but as the density of larvae was about 9 per ml considerable fluctuations in food density must have occurred in the intervals between the daily feeds.

THE CONCENTRATION OF *ISOCHRYSIS GALBANA* IN THE WATER

The most detailed observations were made with *I. galbana* as food, and four to five ml of water for each larva at a temperature of about 21°C. With this degree of dispersion the larvae ate only a small proportion of the available food during 24 hours and the change in cell density during the experiment was insignificant.

The typical relationship between the density of food in the water and the assimilation of ^{32}P is given in Fig. 5 for one series of experiments using larvae of 206 μ.

Seven series of experiments of this type were made in all and the results are plotted together in Fig. 6 by referring each series to a common origin – a cell density of 100 cells

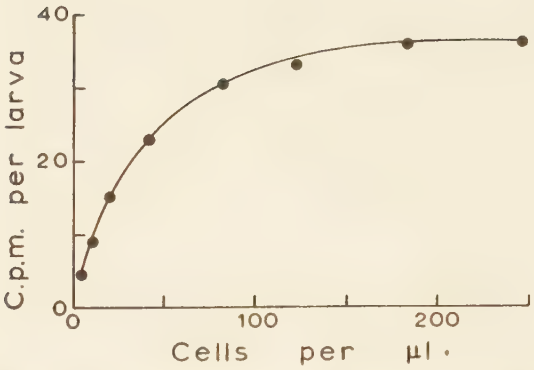

Fig. 5. *The uptake of* ^{32}P *by larvae fed at various densities of* Isochrysis galbana. *The mean length of the larvae at the beginning of the experiment was* 206 μ. *Duration of experiment* 19 *hours.*

per μl. The method of reference was to plot separately for each series the mean radioactivity of the larvae against the density of food on which they had been feeding and to draw a smooth curve through the points – as in Fig. 5. The mean activity obtained with a cell density of 100 cells/μl was read off and taken to equal 100 and the other results were then increased or reduced in proportion. In Fig. 6 the food density has been plotted on a logarithmic scale so that the range could be covered while giving prominence to values below 100 cells per μl, where interest is largely centred.

Four different broods were used in the seven series of experiments and the mean sizes of the larvae at the beginning of the experiments were in the range 206–295 μ. The results show a very consistent relationship between assimilation by the larvae and the density of food in the water, with a rapid increase in assimilation as a low level of food density increases until, at a density of about 50 cells per μl, about 70% of the maximum assimilation is obtained. As the density of food in the water rises above about 25 cells per μl the increase in food assimilated becomes less and less until at densities of over 100 cells per μl the increase in assimilation is very slight for substantial increases in cell density. It is interesting that even at densities up to 1,000 cells per μl no decrease in assimilation was observed.

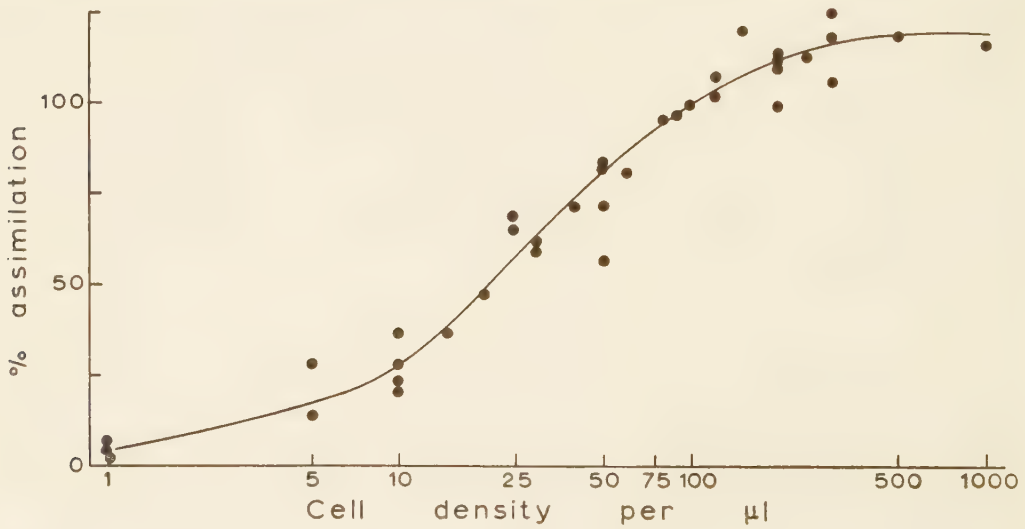

Fig. 6. *The combined results of seven series of experiments measuring the uptake of* ^{32}P *by larvae fed at various densities of* Isochrysis galbana. *The initial mean lengths of the larvae were* 206, 206, 216, 251, 220, 253 and 295 μ.

Similar experiments were made in which growth was used to gauge the reaction of the larvae to various food densities. Because the difference between the growth of larvae fed with 50 *Isochrysis* cells per μl and 100 per μl is not very great, it is desirable for such experiments to last for about two days. A typical result is shown in Fig. 7a, where the growth increment obtained in 39 hours is related to cell densities of 0, 10, 25, 50 and 100 cells per μl for larvae with an initial mean length of 229 μ. Some increase in shell size is made by unfed larvae, presumably by utilising food reserves, but it is only a small amount after the first 24 hours of starvation. In this experiment the shells of the starved larvae grew 8·5 μ in the first 22 hours, but only a further 2·6 μ in the subsequent 17 hours. In this experiment the larvae, cultured in 1 litre beakers, were fed on normal *Isochrysis* and the growth increment increased considerably with additional food up to about 50 cells per μl, but further increase in the food density gave only a small additional size increment. By using the data presented in Fig. 6 an estimate can be made of the relative assimilation at the different cell concentrations. In Fig. 7b, the growth (as measured) is related to the assimilation (as estimated) and demonstrates how closely the cell density in the water, the food assimilated by the larvae and their growth increment are inter-related. In Fig. 7c the estimated assimilation is related to the weight increase calculated from the length-dry weight curve given in Fig. 2. If the increment made in the absence of food is deducted then the remaining weight increment is directly proportional to the calculated assimilation. In some experiments reported later in this paper (Fig. 30) the larvae were fed at the various food concentrations for 24 hours before the experiment began and then no correction was needed for growth in the absence of food.

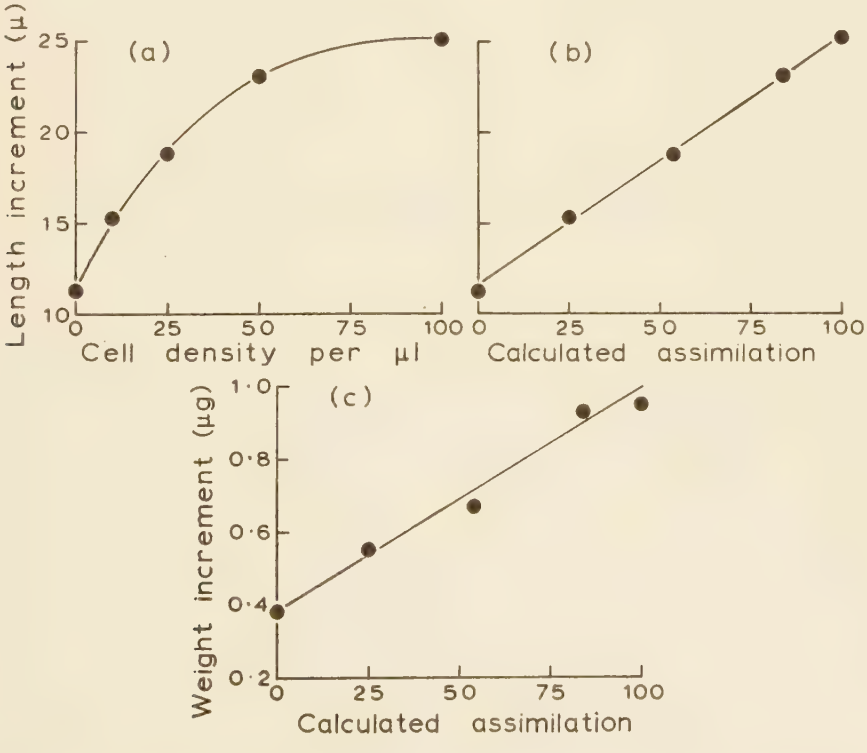

Fig. 7. (a) *The length increment obtained by feeding larvae at various densities of* Isochrysis galbana *for* 39 *hours. Initial length* 228·7 μ.

(b) *The relation between the length increment obtained and the assimilation calculated from Fig.* 6.

(c) *The relation between the calculated assimilation and the length increment obtained converted to a weight increment using Fig.* 2.

The fact that assimilation fails to keep pace with increasing numbers of cells in the water over about 25 cells per μl leads to inquiry into the methods used by the larvae for regulating their food consumption.

Yonge (1926) describes how in feeding larvae food particles are thrown by the large cilia of the velum onto a ciliated tract at the base of the velum where the particles are embedded in mucus and then carried to the mouth. Any surplus material is carried by cilia onto the lobe representing the rudiment of the foot, from which it drops off. The larva has therefore two external mechanisms for dealing with a super-abundance of food in the water – it can either reduce the rate of catching or increase the rate of rejection by the foot rudiment (which is acting in the same manner as the palps of the adult). There is also the possibility that it can vary its digestive efficiency. These aspects of the feeding behaviour have been examined in three experiments in which the larvae have been sufficiently concentrated for an estimate to be made of the number of cells caught. It is assumed that if a cell is caught by the velar cilia it becomes so entangled by mucus that it is removed from the water whether it is actually eaten or subsequently rejected.

In the experiment outlined in Table 1 about two larvae per ml were fed on radioactive *Isochrysis* at four different cell densities. At the end of about 24 hours the larvae were removed for radioactive assay and the density of the cells remaining in the water estimated by haemacytometer counts. From these experiments it is therefore possible to calculate the number of cells assimilated by the larvae from the radioactive assay data and to estimate the cells caught by the larvae, and from this the feeding activity, by calculating the volume swept free (F) from the decrease in the number of cells. The 'volume swept free' in unit time may be defined as:

$$F = V.G.t$$

where V is the volume of medium per animal, G the grazing rate and t unit time. G is given by:

$$G = R - \frac{1}{t} .\log_e \frac{P_1}{P_0}$$

where P_0 is the number of cells per unit volume at the beginning of the experiment, P_1 the number at the end of the experiment, t is the duration of the experiment in days and R the reproductive rate of the food in a control without animals:

$$R = \log_e \frac{P_1}{P_0}$$

This, where determined, was quite small – about 0·1 (Jørgensen, 1943, Cushing, 1958).

Table 1: *The grazing rate and uptake of* ^{32}P *by larvae in* 24 *hours when placed in different densities of* Isochrysis galbana. *Initial length* 219·3 μ. *Temperature* 23–24°C. *Volume* 100 *ml. See also Fig.* 8

The reproductive rate, R, $= \log_e \frac{133}{123} = 0{\cdot}069$							
P_0, cell density/μl	123	123	92	61	61	30	30
P_1, cell density/μl	46	42	43	15	20	8	12
Time, days	1·11	1·16	1·12	1·14	1·16	1·14	1·17
G, daily grazing rate	0·947	0·995	0·742	1·299	1·082	1·228	0·851
V, volume per animal (ml)	0·47	0·36	0·51	0·51	0·53	0·65	1·16
F, volume swept free (ml)	0·445	0·358	0·378	0·663	0·574	0·798	0·988
Counts per minute/larva	26·2	29·0	21·4	17·2	13·8	7·1	6·6
Cells caught/larva/24 hr.	36,411	28,728	25,337	23,251	21,990	14,176	20,555

The weakest part of such experiments is the estimation of the final cell density in those experiments where the food was rather sparse, since unless numerous haemacytometer samples are searched it is difficult to find enough cells to count. If only 100 cells are counted in all, the 95% confidence limits are ±20% of the mean. An attempt to use a photometric method was a failure because of the errors caused by faecal and 'pseudo-faecal' particles.

The results (Table 1, Fig. 8) indicate that the volume swept free increases as the abundance of food decreases – the larvae change their behaviour and feed more actively. It may be that the same volume is filtered at all densities but that when food densities are low it is filtered more efficiently and less food rejected, or it may be that the larvae are filtering more actively at low food densities. In Fig. 8 the volume swept free by a larva in 24 hours is related to the calculated cell density half-way through the experiment, and the estimated volumes swept free, calculated from the upper and lower 95% confidence limits of the final cell counts, are also shown. On the basis of the average figures, the volume swept free was 2–3 times greater at the lower cell densities than at the higher, suggesting that either the actual filtering activity or its efficiency increased with decreasing cell density. The question of digestive efficiency is discussed after the following two experiments have been reported.

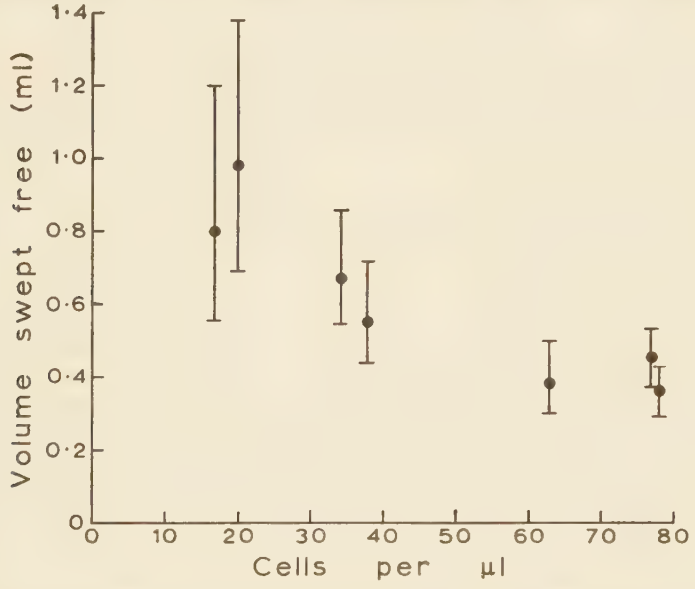

Fig. 8. *The relation between the volume swept free and the mean cell density on which the larvae had been feeding. The limits of the volume swept free for each cell density have been calculated from the* 95% *confidence limits of the final cell density. Initial length of larvae* 219·3 *µ. See also Table* 1.

A similar experiment (Table 2 and Fig. 9) was made using rather higher larval and food densities, but as practically all the food was eaten an estimate of the grazing rate cannot be made. In this experiment growth as well as absorption of ^{32}P was measured, and a close relationship between cells caught, ^{32}P absorption and growth was found (Figs. 9 and 10b), although conditions were varied by altering the concentration of food and the volume of water for each larva. A control with a relatively low density of larvae (7·2 ml per larva) and plenty of food (123 cells per µl) enables a comparison of growth and assimilation to be made with the results obtained in those vessels where the competition for food was much greater. In two of them, with about 5 larvae per ml and 371 and 247 cells per µl initially, growth and assimilation were very similar to those obtained in the controls. In the other experiments food was the severely limiting factor. This comparison demonstrates that it is possible to obtain satisfactory growth with larvae at 5 per ml and several hundred flagellates per µl at the beginning of a 24 hour period, but with more larvae or lower food densities, the grazing rate is sufficient to reduce the food density to levels which restrict growth.

Table 2: *The grazing rate, uptake of ^{32}P, and growth increment by larvae in 24 hours when various numbers were placed in different densities of* Isochrysis galbana. *Initial mean length* 230·8 *μ. Temperature* 21–22°*C, volume* 100 *ml, except for the one with* 1 *larva*/7·2 *ml where it was* 400 *ml*

P_0, cell density/μl	371	371	247	247	123	123	123
P_1, cell density/μl	60	5	13	0	2	0	—
G, daily grazing rate	1·821	4·307	2·944	—	4·119	—	—
V, volume per animal (ml)	0·18	0·08	0·17	0·08	0·16	0·09	7·2
F, volume swept free (ml)	0·33	0·34	0·50	—	0·66	—	—
Counts per minute/larva	88	56	82	44	46	25	86
Cells caught/larva	55,980	29,280	39,780	20,995	19,360	10,947	—
Growth increment (μ)	20·2	18·4	21·1	13·4	7·4	4·3	20·3

In the flask which initially had 371 cells per μl and 0·18 ml per animal, the larvae apparently caught too many cells for the growth increment and ^{32}P absorption recorded. This experiment had the highest density of cells left at the end of it, and it seems probable that these larvae, which had been feeding in cell densities ranging from 371 to 60 per μl, had a high rejection rate. The growth and ^{32}P absorption for this experiment were normally related (Fig. 9) indicating that the relation between assimilation and growth was as previously found.

In a similar experiment with larvae from the same brood on the day after liberation (Table 3), the volume swept free was only about 0·06–0·25 ml and the number of cells eaten was about 12,000 per larva.

In the three series of experiments reported above and in two others in which both cells eaten and the absorption of ^{32}P were measured, the gross efficiency of feeding and digestion together can be estimated by considering the mean number of cells caught and estimating from the radioactivity of the larvae the minimum number of cells which must have been assimilated. In Fig. 10a–e the mean number of cells caught by a larva is related to the mean number assimilated in each of the series of experiments, and regression lines have been drawn in by eye so as to pass through the origins. The quantity of food eaten by the larvae was varied in (a) by changing the density of the larvae, and in (b), (c) and (e) by varying both the larval density and the food density; in (d) the temperature was varied. The deduction from these results is that in these experiments the larvae have generally assimilated a

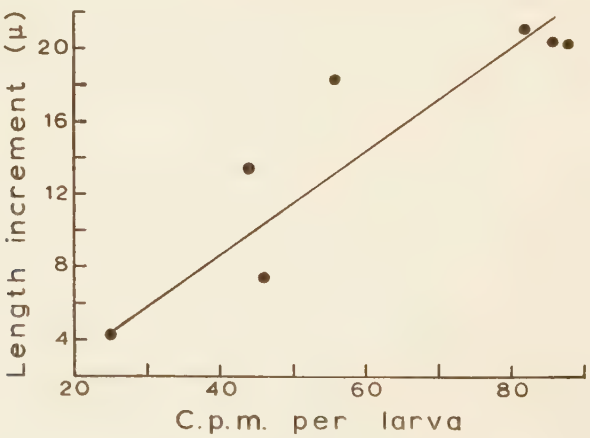

Fig. 9. *The relation between assimilation of ^{32}P and growth when larvae at various densities are fed with various densities of* Isochrysis galbana. *Initial length of larvae was* 230·8 *μ. See also Table* 2.

Table 3: *The grazing rate, uptake of ^{32}P, and growth increment by larvae in 24 hours when various numbers were placed in various densities of* Isochrysis galbana. *Initial mean length* 178·2 *μ. Temperature* 23–24°*C, volume* 100 *ml, except for the one with* 7·1 *ml/larva where it was* 400 *ml. This was the same brood of larvae as used in Table* 2

P_0, cell density/μl	234	234	117	117	117
P_1, cell density/μl	165	110	37	8	—
Time, days	0·95	0·93	0·94	0·94	0·94
G, daily grazing rate	0·368	0·812	1·225	2·854	—
V, volume per animal (ml)	0·17	0·095	0·18	0·087	7·1
F, volume swept free (ml)	0·062	0·077	0·220	0·248	—
Counts per minute/larva	28·3	24·1	22·3	14·6	27·9
Cells caught/larva	11,730	11,780	14,400	9,483	—
Growth increment (μ)	10·8	8·6	9·0	8·6	10·2

constant fraction of the food caught. There is one case (Fig. 10b), already discussed, where the food density was exceptionally high, and the rejection rate apparently rose, but in general, working within the range of cell densities that influence the growth rate and for a given sample of larvae and *Isochrysis* culture, the relation between the cells caught and

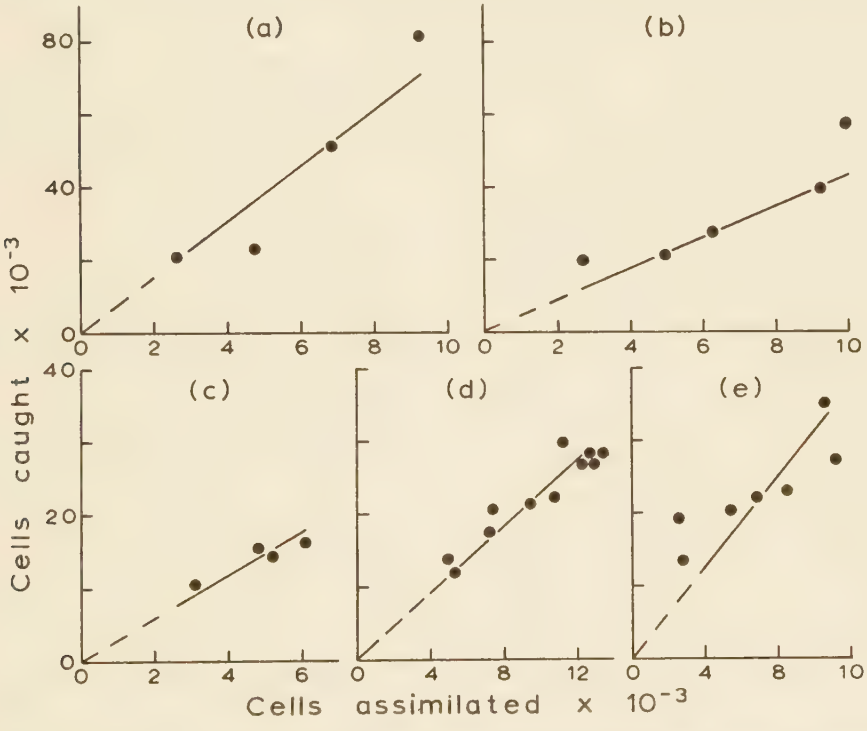

Fig. 10. *The relation between number of cells caught by larvae and the number assimilated, as estimated from the uptake of ^{32}P in five series of experiments.*
(a) *Larvae* 216 μ, 0·2–4·2 *per ml.* Isochrysis 100 *per μl. Duration* 24 *hours.*
(b) *Larvae* 231 μ, *various densities. Various densities of* Isochrysis (*see Fig.* 9 *and Table* 2).
(c) *Larvae* 178 μ, 0·1–11·5 *per ml.* Isochrysis 125–250 *per μl. Duration* 24·5 *hours.*
(d) *Larvae* 220 μ, 2·9–4·2 *per ml.* Isochrysis 132 *per μl. Experiments made at various temperatures* (*see page* 31 *and Fig.* 25).
(*e*) *Larvae* 219 μ, 0·9–2·7 *per ml.* Isochrysis 30–123 *per μl* (*see also Fig.* 8 *and Table* 1).

assimilation is constant. The rejected cells include those that are caught and not eaten and those which are eaten and either not digested or only incompletely digested. The data do not tell us which of these factors was operating or whether the ratio between them varied, and without devising a method of collecting the faeces or observing the number of cells eaten as opposed to caught, this problem cannot be answered.

In Fig. 10 the apparent efficiencies of absorption are respectively 13, 24, 33, 45 and 31%. Marshall and Orr (1955a) estimated that the utilisation of ^{32}P by *Calanus* was over 80% in many experiments and this was confirmed in a shorter series of experiments using C^{14} (1955b). These estimates were made by assaying both the animal at the end of the experiment and the faecal pellets produced, the total of the two observations being the food ingested. Subsequent work has suggested that some allowance should be made for excretion but the error is not very great (Marshall and Orr, 1961). Currie (1962) examined the chlorophyll in the food and the faeces and found that most of the ingested chlorophyll had been at least partially digested. In oyster larvae the gut is very short, which would tend to reduce the digestive efficiency, and also the technique used does not allow of any correction being made for cells which are caught and not eaten.

These observations, using *Isochrysis,* have demonstrated that assimilation by oyster larvae is proportional to cell density up to about 25 cells per μl, while above 50 cells per μl substantial increases in cell density cause only rather small gains in assimilation. The absorption of ^{32}P by larvae of different broods and size-ranges, when fed at various cell densities, bears a very constant relation to that obtained when the larvae are fed at the control standard of 100 cells per μl. Cells caught, growth and assimilation are closely interrelated, while the proportion of cells caught which is assimilated was found, for a given

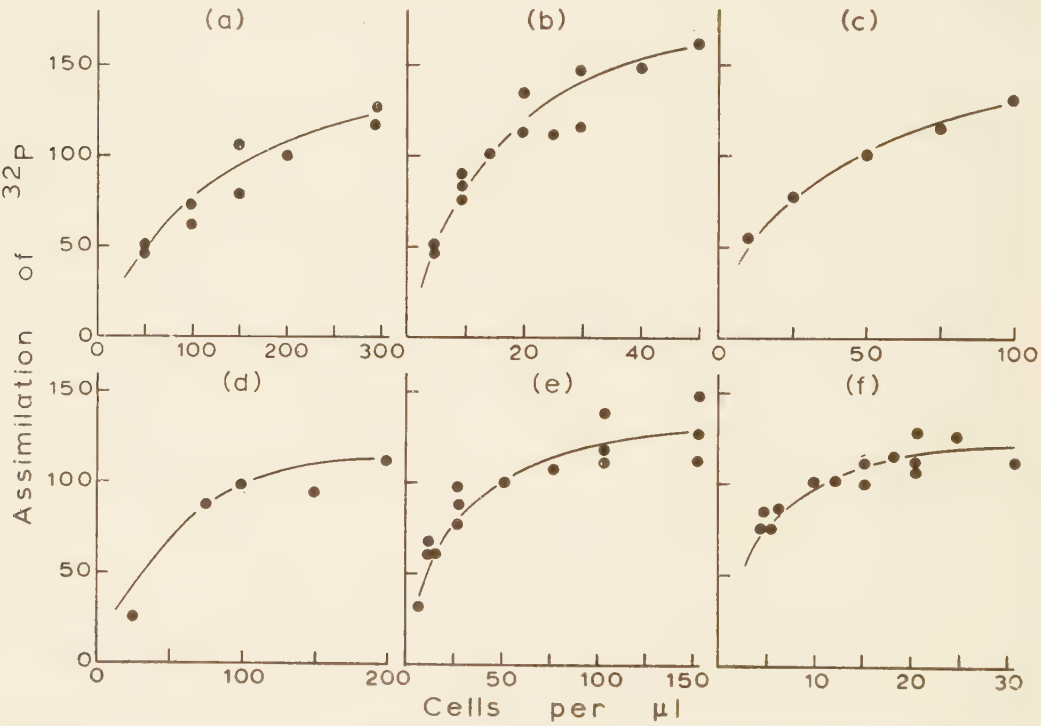

Fig. 11. *The relation between assimilation of ^{32}P in arbitrary units and the cell density on which the larvae were feeding.*

(a) Micromonas minutus. *Two series of experiments. Larvae* 195 *and* 183 μ.
(b) Chlamydomonas subehrenbergii. *Three series of experiments. Larvae* 205, 215 *and* 261 μ.
(c) Chlamydomonas coccoides. *One series of experiments. Larvae* 218 μ.
(d) Dicrateria inornata. *One series of experiments. Larvae* 218 μ.
(e) Phaeodactylum tricornutum. *Three series of experiments. Larvae* 199, 204 *and* 244 μ.
(f) Dunaliella tertiolecta. *Four series of experiments. Larvae* 192, 200, 227 *and* 244 μ.

batch of larvae, to be independent of the level of absorption. Larvae respond to reduced food concentration either by an increase in filtering activity or by improving their filtering efficiency.

THE CONCENTRATION OF SPECIES OTHER THAN *ISOCHRYSIS* IN THE WATER

In the previous section it has been shown how the larvae are very sensitive to different densities of food and that their reaction to it is constant over a wide variety of larval sizes. In this section the results of experiments using seven further algal species, covering a volumetric size range from five times greater to over an order of magnitude smaller than that of *Isochrysis,* are reported. In all cases, with the exception of *Chlorella stigmatophora* Butcher (not illustrated), the relation between ^{32}P absorption and cell density was similar to that obtained with *Isochrysis,* although the position of the inflexion varied (Fig. 11).

The results obtained with *C. stigmatophora* were so strikingly different from those obtained with the other species that they deserve to be discussed separately. *C. stigmatophora* is one of the few plants which, although of suitable size (4–6 μ) and habit, is apparently of little or no value to the larvae of *O. edulis.* Its status as a larval food has recently been discussed at length elsewhere and will not be repeated here (Collyer, 1965, Walne, 1963). On two occasions during the present work larvae were fed on various densities of *Chlorella* (Table 4). In the first series there was no variation in the amount of assimilation over the range tested (25–150 cells per μl), while in the second series there was some increase in assimilation towards the lower cell densities. In the first series 1 c.p.m. was equivalent to 309 cells while in the second series it was equivalent to 72 cells. Taking the results from the 150 cells per μl experiments this is equivalent to digesting 3,990 and 3,020 cells respectively, compared with the 10,000–20,000 cells of *Isochrysis* which would be digested by larvae of this size. It is therefore apparent that the larvae fail to digest this species in the same quantity and manner as the others tested. The recent consideration of the problem in the two papers quoted above has supported the view that the inability of the larvae to utilise this plant is associated with its rigid cell wall, although it should be noted that the presence of a similar cell wall in *Chlamydomonas* does not inhibit the usual relation between ^{32}P absorption and food available (Fig. 11b, c). Although the results with *Chlorella* are unusual, some ^{32}P was absorbed from the cells which, although it was not quantitatively related to the available food, indicates that some assimilation was taking place.

The shape of the curves in Fig. 11 is similar to that previously given for *Isochrysis* (Fig. 5), but the position of the inflexion varies a good deal from species to species. The comparison of the results from different algal species is facilitated by calculating the percentage increase in assimilation obtained when the cell density in the water is increased by

Table 4: *The average absorption of* ^{32}P *(c.p.m.) by larvae from* Chlorella stigmatophora *when fed at various cell densities for about* 24 *hours*

	1st series	2nd series
	Initial larval length (μ)	
Food cells per μl	201·9	217·6
150	12·9	42·5
100	12·3	45·0
75	12·4	43·8
50	11·7	56·8
25	12·4	56·8

a fixed amount. It was found that a convenient level is given by calculating the cell density in the water at which an increase of 5 cells per μl raises the total ^{32}P observed by 2%. With *Isochrysis* this corresponds to 58 cells per μl, a density at which larval assimilation is about 75% of the maximum. Invariably at lower levels the percentage increase is greater and at higher levels, lower. The results are:

Species	*Cell density per* μl	*Volume of* 10^6 *cells in* μl
Micromonas minutus Butcher	132	0·006
Dicrateria inornata Parke	100	0·03
Isochrysis galbana Parke	58	0·1
Chlamydomonas coccoides Butcher	55	0·3
Chlamydomonas subehrenbergii Butcher	42	1·1
Phaeodactylum tricornutum Bohlin	42	0·14
Dunaliella tertiolecta Butcher	25	0·52

All of these species, with the exception of *Chlamydomonas* (which has not been tested), have been shown to be satisfactory food for oyster larvae (Walne, 1956a, 1963), but equivalent volumes of different species are not necessarily equally nutritious (Davis, 1953, Walne, 1963). The comparative cell size quoted in this table is the packed cell volume occupied by 10^6 cells. This has been calculated from observations made by the writer for a number of cultures. The results show clearly (Fig. 12) how the larvae become relatively satiated with food at much lower cell densities if the cells are large than if they are small. When the cell size of the food increases by an order of magnitude, then the cell density in the water required to give a 2% increase in the total food assimilated is halved.

An experiment was made to observe the rate of feeding of the larvae on these various species at densities approximating to the beginning of the plateau in the ^{32}P absorption curves. All the above species were tested except that *Thalassomonas caeca* Butcher was substituted for *Micromonas minutus* and *C. coccoides* was omitted. 10^6 cells of *T. caeca* had a packed cell volume of 0·0036 μl, and this organism was therefore slightly smaller than *M. minutus*. The results (Table 5, Fig. 13) show that the number of cells eaten was linearly related to the mean cell density during the experiment, indicating that the cells in this size range were equally well caught. This may be due to the larvae filtering with constant efficiency over the range of cell size tested (3–10 μ) or, alternatively, to feeding at a higher rate on small cells so as to compensate for the reduced ease with which they are caught.

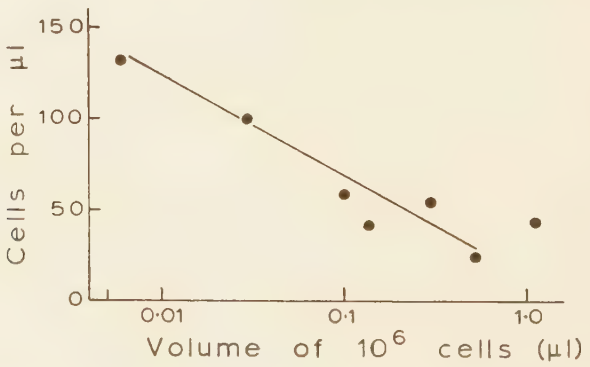

Fig. 12. *The relation between the packed cell volume of* 10^6 *cells of* 7 *species of algae, on a log scale, and the density in the water at which an increase of* 5 *cells per* μl *increases the assimilation of* ^{32}P *by the larvae by* 2%.

Table 5: *The volume swept free in 24 hours by larvae of an initial length of 203 μ, when fed with different species of algae in duplicated experiments*

	T. caeca		*I. galbana*		*C. subehrenbergii*		*D. tertiolecta*		*D. inornata*		*P. tricornutum*	
P_0, cell density/μl	200	200	100	100	30	30	30	30	200	200	140	140
P_{13} cell density/μl	99	75	52	36	24	14	19	24	122	56	73	24
Time, days	0·92	0·94	0·96	0·96	0·98	0·98	1·02	1·02	1·04	1·05	1·06	1·07
G, Daily grazing rate	0·77	1·06	0·68	1·06	0·23	0·78	0·46	0·22	0·47	1·21	0·69	1·53
V, volume per animal (ml)	0·27	0·29	0·25	0·31	0·27	0·29	0·26	0·27	0·43	0·39	0·62	0·30
F, volume swept free (ml)	0·21	0·31	0·17	0·33	0·06	0·23	0·12	0·06	0·20	0·47	0·43	0·46
Cells eaten/larva/24 hours (Thousands)	27	36	12	20	1·6	4·6	2·9	1·6	34	56	41	35

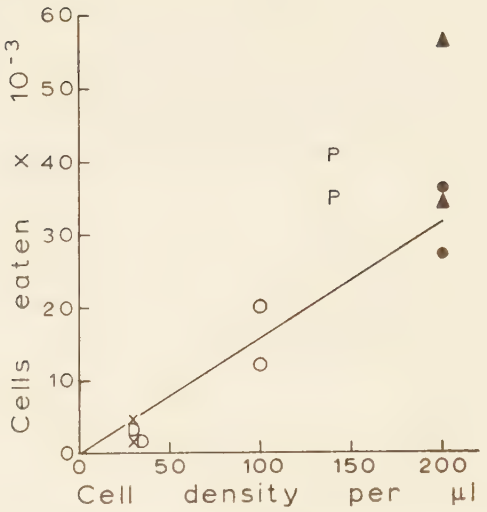

Fig. 13. *The relation between the number of cells caught and the mean density of cells in the water when larvae were fed 6 species of algae at densities at which maximum feeding was expected. See also Table 5.*

● Thalassomonas caeca
O Isochrysis galbana
▲ Dicrateria inornata
X Chlamydomonas subehrenbergii
D Dunaliella tertiolecta
P Phaeodactylum tricornutum

FOOD CONSUMPTION AND GROWTH OF LARVAE

In this section the various observations made during this study on the amount of food eaten and digested by larvae are brought together and related to the size of the larvae and to the average growth rate under those conditions. From these observations it is possible to relate the food consumed to the energy requirements of the larvae for growth and for respiration.

FEEDING BEFORE LIBERATION

Although larvae which are being brooded in the mantle cavity have been observed to have particulate material in their stomachs, it is difficult to suppose that feeding can be important before they are released into the water. At the time of liberation larvae still have considerable glycogen reserves (Collyer, 1957) and are capable of further growth in size in the absence of food. When the shell length is in the range 130 150 μ differentiation is not sufficiently advanced for feeding to be possible, but in the range 150–170 μ they appear to be sufficiently differentiated although in the field they are not normally liberated until 170 μ or larger (Walne, 1956a).

The influence of food on the growth of young larvae (150–170 μ) was studied in 15 experiments, using six broods which had been removed from their parents at an early stage of development and cultured in glass vessels. When they began to develop shells small numbers of larvae were added to beakers containing water enriched with 75–100 cells per μl of *I. galbana*. The controls did not contain added food and the water temperature was 22 23° C. By setting up at the same time a number of unfed controls and feeding these on successive

Table 6: *The increase in length obtained by feeding embryos with* Isochrysis galbana *shortly before the normal stage of liberation is reached. Single experiments were made with broods* 5 *and* 6; *with broods* 1–4 *the experiments were in duplicate*

Brood of larvae	*Initial length* (μ)	*Final length* (μ) *Unfed*	*Fed*	*Increment due to feeding* (μ)
1	164·6	170·6	174·5	+ 3·9
	170·6	182·9	186·4	+ 3·5
2	157·5	165·4	169·1	+ 3·7
	165·4	179·1	184·9	+ 5·8
3	155·0	166·3	165·4	− 0·9
	166·3	175·5	185·5	+10·0
4	158·8	169·2	174·2	+ 5·0
	169·2	176·8	180·2	+ 3·4
	176·8	180·1	186·6	+ 6·5
5	140·0	158·8	166·2	+ 7·4
	158·8	176·4	179·2	+ 2·8
	176·4	179·8	186·8	+ 7·0
6	155·7	170·8	172·0	+ 1·2
	170·8	182·0	184·6	+ 2·6
	182·0	190·4	195·2	+ 4·8

days, it was possible to compare the effect of the introduction of food at various stages in development. Samples of larvae were preserved for measuring after feeding for 24 hours.

The results, which are set out in Table 6, show that in only one out of 15 experiments was no extra growth obtained when the larvae were fed; in the other 14 better growth was obtained with the fed larvae than with the unfed. The results can be conveniently compared by plotting the ratio of the instantaneous relative growth rate of the fed larvae (K_F) to that of the unfed (K_O) against the mean size at the beginning of the period:

$$K_F \text{ or } K_O = \frac{\log_e L_2 - \log_e L_1}{t}$$

where L_1 and L_2 are the length of the larvae at the beginning and end of the experiment and t the duration in days; K has been used by Dehnel (1955) to compare the growth of gastropod embryos at various temperatures.

If $\frac{K_F}{K_O}$ is about 1 then feeding has had no effect; if greater than 1 then feeding has accelerated growth (Fig. 14). This method of treating the data also allows for the fact that some broods of larvae grew faster than others. The results show that, in larvae with a mean length of about 157 μ or more, the rate of shell growth was enhanced in the following 24 hours by the presence of food. There was a tendency for the ratio to be greater as the size

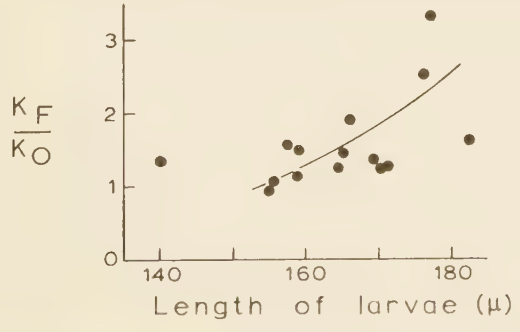

Fig. 14. *The ratio of the specific growth rate of fed* (K_F) *and unfed* (K_O) *larvae plotted against the mean length of the larvae at the beginning of the* 24 *hour period.*

of the larvae increased. In one case where the initial size was about 140 μ – it was difficult to measure exactly by transmitted light – the larvae apparently fed during the subsequent 24 hour period. These larvae were extremely vigorous (brood 5, Table 6), growing about 20 μ a day, and it is possible that they were differentiating sufficiently quickly to start feeding towards the end of the 24 hour period.

These results demonstrate that the larvae are able to feed to the benefit of their growth rate when they are at a size at which they are normally still within the mantle cavity. Whether or not delay in commencing to feed, due to prolonged retention by the parent, is harmful is unknown. The amount of food consumed at this stage is small. The number of cells eaten was determined for three broods over a period of about 43 hours. The initial sizes were 165, 158 and 155 μ respectively (broods 1, 2 and 3 respectively of Table 6), duplicate determinations being made of brood 1 and single determinations of broods 2 and 3. The reproductive rate of the alga was determined in a control experiment without larvae. The data, set out in Table 7, show that F, the volume swept free/larva/24 hours, was 0·02–0·06 ml. This is roughly 10% of that obtained with larger larvae (page 11).

Table 7: *Grazing by small oyster larvae at* 24°*C. The experiment lasted for* 1·8 *days*

R, reproductive rate of alga per day, $= \frac{1}{1{\cdot}8} \cdot \log_e \frac{110}{85} - 0{\cdot}143$

Brood	1	1	2	3
Initial mean length (μ)	164·6	164·6	157·5	155·0
Final mean length (μ)	182·0	183·6	180·8	178·4
P_0, cell density/μl	85	85	85	85
P_1, cell density/μl	40	38	68	66
G, daily grazing rate	0·562	0·590	0·267	0·284
V, volume per animal (ml)	0·131	0·130	0·149	0·213
F, volume swept free (ml)	0·074	0·077	0·040	0·060
Cells caught/larva/day	5,109	5,261	3,180	4,790

CONSUMPTION OF *I. GALBANA*

In experiments using *I. galbana* marked with ^{32}P it has been the practice to make, in each series, a control experiment with a cell density of 100 cells/μl at a temperature of 21–22°C with about 4 ml of water for each larva. The radioactive count of the cells was determined at the beginning and end of the experiment and from this the mean number of cells equivalent to 1 c.p.m. was calculated. From the larval assay the minimum number of cells which the larvae must have assimilated to acquire that activity can then be estimated. The experiments lasted for about 24 hours and, by proportion, the minimum number of cells assimilated per larva per 24 hours was calculated. This figure is independent of the ^{32}P in the algal cells and can therefore be used to compare the assimilation by different batches of larvae at widely different times. It does, however, assume that all the ^{32}P assimilated during the experimental period has been retained and none lost by excretion. Observation has shown that the loss in the period after the end of the experiment is only a few per cent per day – considerably less than that recorded for *Calanus* (Marshall and Orr, 1961) – and the error therefore is not very great.

The number of cells assimilated under these conditions has been determined in 32 experiments over a period of three years and in Fig. 15 it is related to the mean length of the

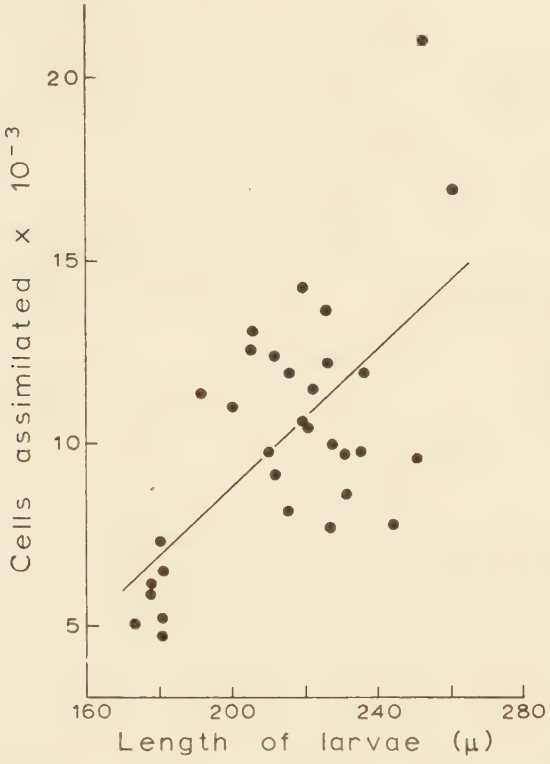

Fig. 15. *The calculated minimum number of cells assimilated by larvae in* 24 *hours when feeding on* Isochrysis galbana *at* 100 *cells per* μl, *related to the length of the larvae.*

larvae at the beginning of the experiment. The regression of cells assimilated on larval length has been calculated and is drawn in on the figure. The regression indicates an increase of about $2\frac{1}{2}$ times in the assimilation during the planktonic phase, the results obtained having a scatter of about ± 30–50% about the regression. Although the scatter of the results is partly due to experimental errors in estimating cell numbers and radioactivity, there are also more fundamental causes of variation. The efficiency of assimilation may vary with the degree to which the ^{32}P is bound in the *Isochrysis* cell. Rice (1953) has shown how, as *Phaeodactylum* cultures age, the proportion of labile phosphorus decreases; hence, it is possible that old cultures are not so readily assimilated. Conover (1956) has shown that old cultures are less readily grazed by *Acartia,* while *Calanus* is not so efficient at using old cultures of *Lauderia* (Marshall and Orr, 1955a). It is therefore possible that some of the variations were due to the use of cultures of different ages. It is also probable that there will have been some differences in the sizes of the cells in the different cultures.

The results are related to the mean initial size of the larvae and, although only populations showing good growth were used, it can be expected that from time to time there were differences in the proportion of slow-growing individuals. The considerable variation which can occur within a population was demonstrated by assaying the individual larvae from a feeding experiment. Larvae with an initial mean size of 236 μ (range 170–260 μ) were used. As these had been liberated for only 4 days, they were growing well and this was confirmed in a sample set up in a 1 litre beaker at the same time as the experiment, which made an average growth increment of 14·4 microns in 24 hours.

At the end of 22 hours the larvae were prepared for assay by picking out 42 larvae at random, measuring, and then counting individually. The 42 larvae had a mean count of 176·0 c.p.m. while a replicate experiment in which 71 larvae were pooled gave 176·9 c.p.m., indicating that the 42 larvae were probably a random sample of the population. The results,

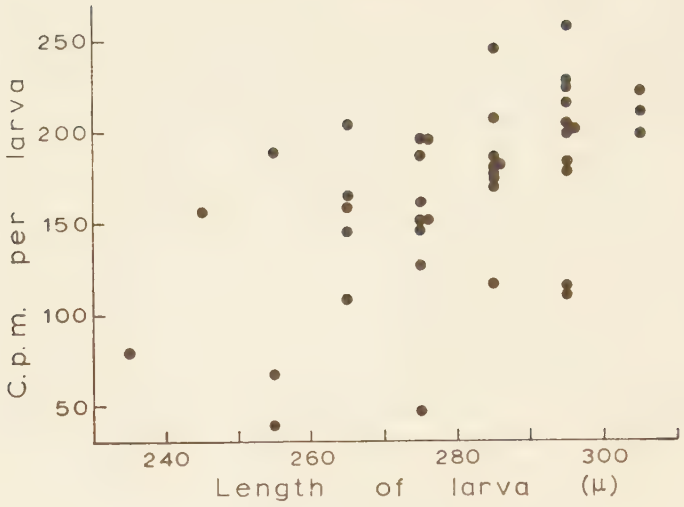

Fig. 16. *The uptake of* ^{32}P *from* Isochrysis *by individual larvae in a brood, related to the length of the larvae.*

given in Fig. 16 where the c.p.m. for each of the individually counted larvae is plotted against its length at the end of the experiment, show a considerable scatter with a trend towards more assimilation by the larger larvae. There were a few larvae which gave very low counts; they were probably moribund. When the samples of larvae for assay were examined under the microscope, specimens were seen from time to time which were of poor colour and with thin tissues within the shell. Such larvae were probably moribund, but it was thought inadvisable to exclude them on such a subjective basis; however, variations in the proportion of these larvae in the population would have an effect on the mean values.

The dry cell weight of *Isochrysis* has been determined by Collyer (1965) for a number of Conway-grown cultures. The results of six cultures gave the mean dry weight of 10^6 cells as 16 μg. The full analysis indicated that the 16 μg contained about 7 μg organic carbon, that is 1 mg carbon was contained in 2·3 mg dry organic matter – a common ratio in marine phytoplankton (Cushing et al., 1958). Using this information, the regression of the estimated minimum cell assimilation in Fig. 16, and the length/dry weight relationship of the larvae in Fig. 2, it is possible to interrelate feeding and size on a dry weight basis. It should be remembered that experiments have shown that the larvae can catch two to four times as many cells as the total given by the assimilation data (Fig. 10) and it is possible that the

Table 8: *The number and dry weight of* Isochrysis galbana *cells assimilated in* 24 *hours by larvae of various lengths*

Length of larvae (μ)	*Dry weight organic matter of larvae* (μg)	*Daily food intake*		*Weight of cells eaten daily, as* % *of larval dry weight*
		As cells	*As dry weight of cells* (μg)	
170	0·17	5,960	0·095	56
180	0·20	6,910	0·111	55
200	0·31	8,810	0·140	45
220	0·43	10,710	0·171	40
240	0·60	12,610	0·202	34
260	0·80	14,510	0·232	29

ratio of phosphorus to the constituents of the assimilated food may not be constant, although, as it has been established that the absorption of ^{32}P from algal cells and the growth of larvae are closely related, it is probable that the absorption of other components will be in constant proportion to the assimilation of ^{32}P.

The food intake, on a dry weight basis, is related to the length and dry weight of larvae in Table 8. According to the ^{32}P absorption figures, the daily food intake of oyster larvae declines from about 56% of its own dry weight of organic matter to 29% during the planktonic stage. In terms of carbon assimilation the figures are about 25% and 13% respectively. Menzel and Ryther (1961) find that zooplankton requires to assimilate carbon equal to 10–12% of its dry weight per day. It is to be expected that embryonic forms like oyster larvae with a high specific growth rate will have higher requirements.

CONSUMPTION OF SPECIES OTHER THAN *ISOCHRYSIS*

The experiments in which the assimilation of ^{32}P from various densities of seven species of algae was measured also yield data on the minimum number of cells digested. This has been calculated in the same manner as used for *Isochrysis* in the previous section. To obtain comparable results between species it is necessary to compare the feeding activity of the larvae at comparable cell densities of the different algal species. This has been done by using the cell density at which an increase of five cells/μl in the water gives a 2% increase in assimilation for each of the species (see page 16).

The results of these calculations are set out in Table 9 and show clearly the decreasing number of cells digested as the food species increase in size. Quite small larvae will assimilate 20,000 cells of flagellates 2–3 microns in size per day, falling to a fifth or less of that number with cells 7–9 microns in size. From the volumetric size data obtained from the observed volumes of packed cells, the minimum volume digested lies in the range of $0{\cdot}01$–$0{\cdot}38 \times 10^{-2}$ μl packed cells/day/larva. Taking the specific gravity of the cells as rather greater than that of seawater, say 1·030, and the dry weight as about 22% of the wet weight (Cushing et al., 1958), the dry weight digested is about 0·23–0·86 μg/larva/day. This is in reasonable agreement with the estimate made from other data for *I. galbana* (Table 8). There was a greater

Table 9: *The minimum number of cells assimilated when larvae are fed at the cell density at which an increase of 5 cells/μl gives a 2% increase in the ^{32}P assimilated*

Food organism	*Larval length* (μ)	*Feeding concentration of cells No./μl*	*Number of cells digested/24 hours*	*Volume of cells digested/24 hours* $\times 10^{-2}$ (μl)
Micromonas minutus	183·4	132	22,800	0·014
	195·4	132	16,380	0·010
Dicrateria inornata	261·0	100	10,400	0·031
Isochrysis galbana	200·0	58	7,050	0·070
Chlamydomonas coccoides	217·6	55	3,840	0·115
Chlamydomonas subehrenbergii	205·2	42	3,000	0·330
	215·5	42	3,510	0·385
	261·0	42	2,610	0·287
Phaeodactylum tricornutum	198·6	42	3,240	0·136
	203·7	42	3,410	0·143
	243·9	42	4,500	0·189
Dunaliella tertiolecta	191·9	25	438	0·023
	200·4	25	438	0·023
	226·9	25	1,380	0·072
	244·0	25	1,180	0·061

agreement within a species than between species, which suggests either that the cell volumes used were not quite comparable or that the feeding levels selected for each species were not fully comparable. Collyer (1965) has shown that there are considerable differences in the amount of water in cells of various species of algae and also that it may vary considerably within a species under different culture conditions.

GROWTH OF LARVAE WHEN FED WITH *ISOCHRYSIS GALBANA*

During this study the growth of a number of broods of larvae has been observed when cultured under standard conditions. The observations were made in vessels holding 400–1,000 ml of filtered seawater with sufficient culture added to give a density of 100 cells per μl. The temperature was in the range 21–23°C and the experiments lasted for 24 hours. The data for these numerous experiments can be conveniently compared by plotting the mean length of the population at the beginning of the experiment against the mean length at the end (Fig. 17). This technique, which gives a straight line relationship, is sometimes known as the 'Walford plot' and is commonly used in investigations on the growth rate of fish. It is a convenient method of relating the growth rate of different batches of larvae and can also be used to follow a given population for a number of days. In fish populations the plotted line intercepts the 45° axis (line of no growth) at the asymptotic length. In the case of oyster larvae the interception appears to have no theoretical significance.

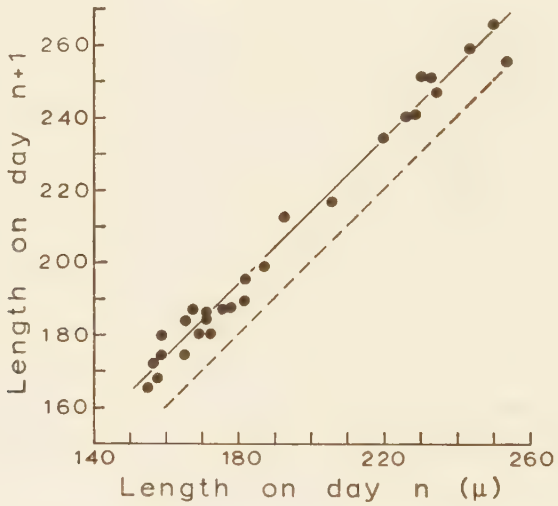

Fig. 17. *The mean length of larvae on day n related to the length on day n + 1 when feeding on* Isochrysis *at 100 cells per ml. The pecked line is the line of no growth.*

The data used in Fig. 17 are from (a) the controls in comparative feeding experiments (Walne, 1963), (b) similar experiments with other broods of larvae, and (c) the fed controls in the observations on the feeding of early larvae reported on page 19 of this paper. These combined data show clearly that a uniform growth response was obtained from larvae, varying in initial length from 155 to 255 μ, when cultured under constant conditions in the presence of an abundance of food.

From these data a growth curve can be constructed (Fig. 18) which shows a linear relation between time and mean length and an exponential relation with weight. Fig. 18 indicates that growth from 175 to 250 μ took about 5·5–6 days. This may be compared with the estimates made from the completely independent set of data obtained in the 48 hour temperature experiments (p. 32), which were 5·9 days at 23°C and 8·4 days at 20·0°C.

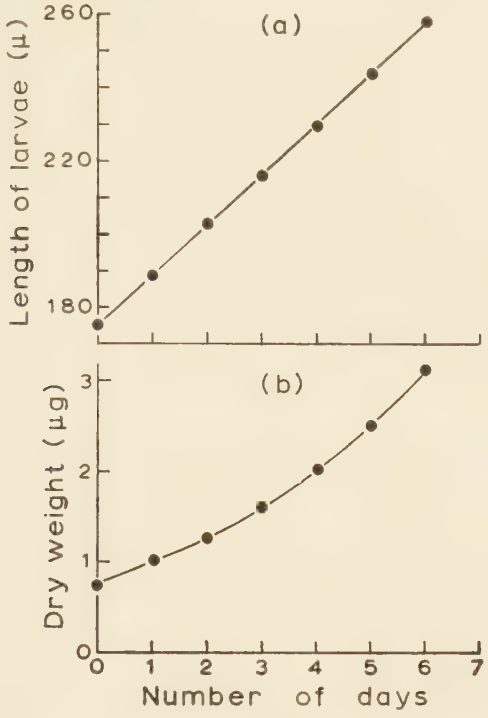

Fig. 18. *A growth curve for* (a) *length and* (b) *dry weight for larvae grown under standard conditions. Calculated from the data given in Fig.* 17.

THE INFLUENCE OF TEMPERATURE ON DEVELOPMENT, FEEDING AND GROWTH

NATURAL breeding populations of *O. edulis* are found in the Mediterranean, on the Atlantic coast of Europe from Morocco to the Shetlands, and on the coasts of countries bordering the North Sea, and a study of the literature, outlined below, gives the temperature at which some of the more important populations breed.

Italy		
Gulf of Taranto (Cerruti, 1941)	26–27°C	
Jugoslavia		
Istria (Nikolić and Stojnić, 1962)	24–26°C	
Spain		
Ria de Vigo (Andreu and Arte, 1955)	16·3°C	Mean for June
France		
R. Auray (Marteil, 1960)	16·5–20·0°C	Average for 23 years
R. Crach (Marteil, 1960)	17·1–22·4°C	Monthly average 1955
Holland		
Oosterschelde (Korringa, 1941)	19·5°C	Average for 19 years; only occasionally exceeds 20°C.
England		
R. Fal (Orton, 1926)	16·5–18·5°C	For 1925. Described as 'more than normally warm'
R. Fal (Orton, 1936)	14·5–17·3°C	For 1927
R. Crouch (Knight-Jones, 1952; Waugh, 1957)	18–23°C	For 1948–54
R. Blackwater (Orton, 1936)	15·0–19·5°C	For 1927. Described as 'a dull and wet summer'

Norway		
Oyster Pols (Gaarder & Bjerkan, 1934)	22–27°C	Exceptional conditions due to a surface freshwater layer

From this summary it is clear that the 10° range of 16–26°C covers the temperature at which the majority of larvae are liberated, and the work reported in this section is mainly concerned with the effect of variations within this range.

Korringa (1957) discussed the problem of possible physiological races and concluded that it is probable that some populations will breed at temperatures as low as 13° C. Similarly Stauber (1950) has speculated on the possibility of physiological races of *Crassostrea virginica,* but so far the necessary experiments have not been made to find out whether warm-water forms fail to breed if they are cultured from early larval stages at reduced temperatures.

THE INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF LARVAE BEFORE LIBERATION

The duration of development within the mantle cavity was estimated by Orton (1926, 1936, 1937a) at 7–10 days by opening weekly oyster samples from natural beds. Erdmann (1935) found that oysters kept in tanks required 18 days at 13–14°C and 6–8 days at 23°C.

Early embryos were obtained by opening a number of oysters until a gravid individual was found. The embryos were then pipetted into a beaker of water and washed free from debris by decanting and replacing the supernatant water several times. A few thousand were added to glass beakers containing 1 litre of filtered seawater to which the standard dose of penicillin and streptomycin had been added. The beakers were stood in water baths, and, until the larvae swam up off the bottom, the water was changed occasionally by decanting and refilling with water of the same temperature. No food was added. A sample of the larvae from each experiment was examined on alternate days and classified according to one of the following stages:

I Fertilised egg.
II Two to about 64 cells; individual cells easily visible.
III Non-motile and somewhat irregular in outline.
IV Regular outline, ciliated and rotating steadily. Not swimming up off the bottom.
V First gleam of shell to be seen by reflected light and velum starting to develop. Not swimming up off the bottom.
VI Shell about 100 μ; velum well-developed, but embryo still opaque. Starting to swim off the bottom.
VII Shell 130–140 μ. Shell unable to cover velum.
VIII Shell 140–150 μ. Many larvae swimming up. Embryo still rather opaque.
IX Shell 150–160 μ.
X Shell 160–170 μ. Fully developed 'D' larva.

This scheme, which is based on that given by Orton (1937b), gives a readily recognisable series of stages which obviously differ both as regards structure and behaviour.

After stage VI the shell could be measured by transmitted light and samples were measured periodically until the mean size of the population exceeded 160 μ, when the experiment was stopped. At this size the larvae have fully developed into their planktonic form, the shell can be completely closed to cover the animal, and then, or soon after, feeding starts, although they are not normally released into the water until they are 170–180 μ. From graphs of the mean size plotted against time for each temperature, the number of days taken to grow to 160 μ was read off, and this was used as an index of the rate of development at the various temperatures.

In a preliminary experiment two broods of very early embryos were obtained: 4- and 8-cell stages respectively. These two broods were cultured at 22–23° C and the 4-cell embryos took about six hours to reach the 16-cell stage, while the 8-cell embryos reached this stage in three hours. After 24 hours both batches were stage III embryos.

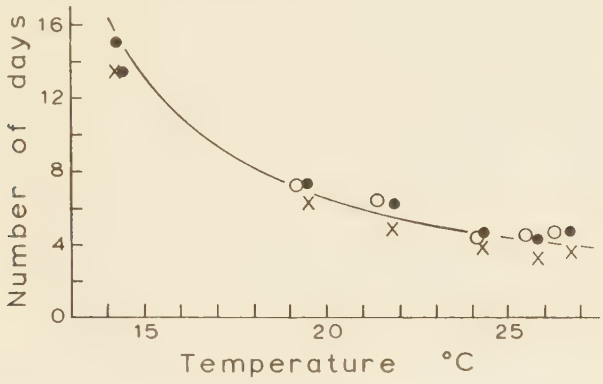

Fig. 19. *The time taken for* 3 *broods of larvae to grow from early cleavage to a mean shell length of* 160 μ *at various temperatures.*
● *Brood A* × *Brood B* o *Brood C*

The development of three broods of embryos was followed in greater detail at six different temperatures. Two (broods A and B) were in stage II (16–32 cells) when obtained, while the third (brood C) was in stage III. From the rate of development of the two stage II broods and the results outlined in the preceding paragraph, it was concluded that the stage III brood was approximately 24 hours older than those in stage II, and the data from these embryos have been weighted by adding 24 hours to bring the three batches into line. The time taken for these three broods to develop to 160 μ is related to temperature in Fig. 19. The curve which has been fitted is that for the formula

$$x \cdot y = K$$

where x is the time in days to reach 160 μ, y is the temperature in °C above biological zero, and K = 66·6. A number of trials showed that the best fit was given on the assumption that biological zero was 10°C. This relationship between temperature and rate of development, sometimes known as thermal summation, has been widely used elsewhere, for example, in dealing with fish eggs (see Simpson in Graham, 1956). The curve given also corresponds in the range 16–26°C to a Q_{10}* of 2·78 – a very commonly found figure.

From these results it can be deduced that development from early cleavage to a shell length of 160 μ takes seven days at 19–20°C. Since early cleavage stages will take only a few hours to develop after fertilisation, this period of seven days may be regarded as commencing at fertilisation and, as 170–180 μ is the usual size at liberation, a further one to two days should be added to give the full period of development within the mantle cavity. These data therefore suggest that at a temperature of 19–20°C development takes about eight days, at 16–17°C about 12 days, while at 22–23°C it takes about six days. The increase in the rate of development with increasing temperature is rather small over about 21°C, and the differences are negligible in the range 24–27°C. An examination of the Q_{10} calculated for 2°C intervals from 15–27°C (Table 10) shows high values in the 15–19°C range, decreasing sharply to about 2 at 21°C. The differences in rate with increasing temperature are very small in the range 24–27°C – at 25°C the rate is only equivalent to 60% increase for a 10° rise in temperature. It is at the lower end of the temperature scale that many larvae are brooded, and it is clear that they respond to very small changes of temperature. Dehnel (1955) has shown that a rather similar situation occurs with the larvae of several species of gastropods, and the dependence on temperature is more extreme at the lower end of the temperature range during the embryonic development of a number of species of barnacles (Patel and Crisp, 1960a).

* Q_{10} is the factor by which a reaction velocity is increased for a rise in temperature of 10°C (Prosser and Brown, 1961).

Table 10: *The Q_{10} values over several temperature intervals for* (a) *the rate of development from early cleavage to a shell length of* 160 μ, (b) *the rate of development from* 175 μ *to* 250 μ, (c) *the assimilation of* ^{32}P *from* Isochrysis galbana

Temperature interval °C	*a*	*b*	*c*
15–17	26·9	—	17·7
17–19	6·5	11·0	8·5
19–21	2·3	8·3	3·7
21–23	2·2	4·9	2·5
23–25	1·8	2·5	1·7
25–27	1·6	—	1·7
17–25	2·9	4·3	2·4

The rate of development of the various stages is related to temperature in Fig. 20. The method of obtaining a linear relation between arbitrarily chosen stages and time is that used for barnacle development by Patel and Crisp (1960a). The ordinate scale was constructed by selecting an experiment made at a temperature near the middle of the range (19·5°C in this case), and arranging the position of the stages of development on the scale in proportion to the percentage of the total time taken to develop to embryos measuring 160 μ (stage X). This scale gives a straight line with the data for other temperatures, although the slope differs according to the different rates of development. The fact that the same scale gives a straight line relationship for all temperatures demonstrates that the proportion of the total time spent in each of the stages is the same at all the temperatures tested.

The experiments made with the two broods obtained in early cleavage were maintained until growth ceased, in order to investigate whether those reared at a lower temperature would grow to a larger size than those reared at a higher temperature, as has been reported for salmon fry (Gray, 1928), and the barnacle *Elminius modestus* (Patel and Crisp, 1960b). Fifty larvae were measured from each vessel but no systematic trend in the results could be seen. Although it may be disguised by the inaccuracy of the mean due to the rather wide range of sizes present, it should have been detected if it was of the same order as that reported for *Elminius*. Loosanoff (1959), investigating a series of data for *Venus mercenaria*,

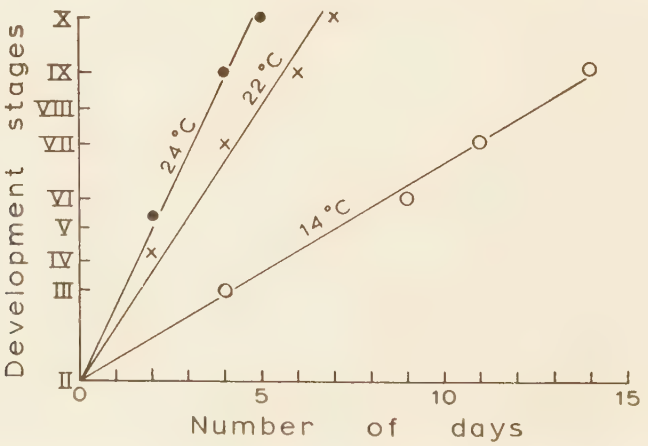

Fig. 20. *The time taken for a brood of larvae to pass through various developmental stages at various temperatures.*

found no relationship between temperature and size at setting, but in that case the larvae were feeding and could therefore possibly compensate for the increased rate of development due to temperature.

The larvae from the brood obtained in stage III and reared at 14·3°C were maintained in culture until metamorphosis. After passing through stage X (160–170 μ), *Isochrysis galbana* was added as food. These larvae reached 160 μ after 12·5 days, and were first fed on the 13th day. By the 25th day the larvae, well pigmented, had reached a maximum size of 260 μ. On the 38th day eyed larvae were present and a few spat were found on the 49th day. On the 58th day, when the experiment was terminated, numerous spat were found and eyed larvae were still swimming. These observations demonstrate that embryos obtained from a river Blackwater oyster and spawned at 20°C can be successfully reared through to metamorphosis without undue mortality at a temperature of 14–15°C, which is well below the normal summer temperature. This result throws some doubt on the theory of the existence of physiological races of *O. edulis* unless it can be demonstrated that there is a physiological block to spawning at such temperatures. This seems unlikely, for Dr. R. H. Millar has found (personal communication) Brittany oysters brooding larvae at temperatures of 14·5–16·0°C in Loch Ryan (S.W. Scotland) in June of the year after relaying.

The fact that these and other early embryos have been successfully reared through to metamorphosis at several different temperatures demonstrates conclusively that the function of the brood mother oyster is to provide protection and suitable physical conditions, and that no essential nutrient is provided after the egg is laid.

THE INFLUENCE OF TEMPERATURE ON THE FEEDING AND GROWTH OF THE LARVAE AFTER LIBERATION

The extent to which raising the temperature would increase the food assimilated by oyster larvae was investigated by feeding radioactive *Isochrysis galbana* to four different broods of larvae in five series of experiments. The experiments were arranged in the usual way in 400 ml flasks. The results of the five series of experiments are shown together in Fig. 21 by referring each series to a common origin by the method used in comparing the concentration of food in the water (page 8). In this case the value for 20·0°C was taken to be 100. The data show a close agreement among the four broods of larvae in the range 15–25°C, but above 25°C the scatter was more pronounced. The points lying above the trend line in this region all come from the one brood which was tested twice, while the other points – which indicate no increase in feeding rate come from three other broods, and it may be that these

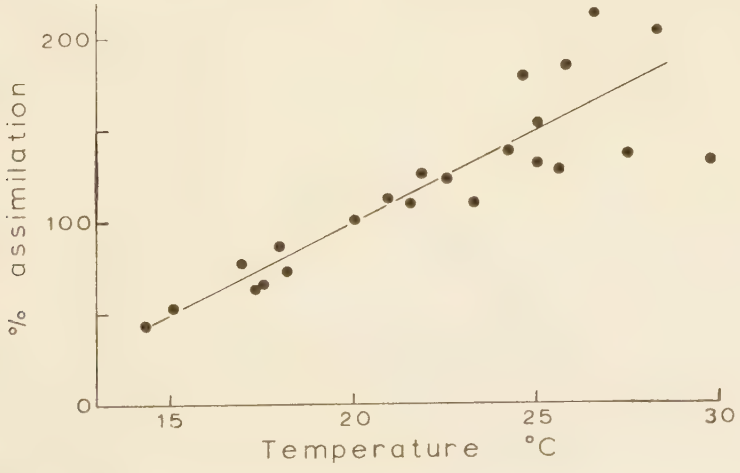

Fig. 21. *The assimilation of* ^{32}P *by the larvae from* Isochrysis galbana *at various temperatures.*

were not so tolerant of the high temperature. The Q_{10} of the assimilation, calculated for 2°C intervals from 15–27°C, is given in Table 10, column c.

The influence of temperature on the growth of larvae was examined in detail in seven series of experiments using larvae with the initial sizes varying from 172·1 to 257·6 microns. In each series six replicate beakers, each holding 1 litre of seawater with *I. galbana* at 100 cells per μl and standard antibiotics, were set up with about 200 larvae in each. The beakers were placed in water baths and the contents gently stirred by aeration. After 48 hours the larvae were killed and the shell length of a random sample of about 100, when the initial size was small, and 200 when large, was measured from each beaker. The temperature range tested varied slightly between each series but always covered the range 15–26°C.

A typical result, shown in Fig. 22, indicates that there is a straight line relationship between the mean length of the population after 48 hours and the temperature. For each series therefore a straight line relationship has been fitted by calculating the regression of temperature on length. From each of these seven curves the length increment at four standard temperatures – 17·0, 20·0, 23·0 and 26·0°C – have been read off. At each of these standard temperatures a plot has been made of the initial length of the larvae against the length of 48 hours later as read from the fitted curve. This is again the 'Walford plot' as applied above (p. 24). The plots (Fig. 23) can then be used to calculate growth rate curves at these four temperatures (Fig. 24a). From these curves the effect of temperature on the duration of the planktonic phase can be estimated (Fig. 24b). The time taken to grow from 175 to 250 μ has been taken for the preparation of this estimate as larvae are usually 170–190 μ when released and, in the field, become eyed somewhere in the range 240–300 μ (Waugh, 1957). The relative speed of development given by this figure agrees with the estimates based on field observations of the duration of the pelagic phase made by Korringa (1941) and Waugh (1957). The data given in these two papers are entered on Fig. 24b.

The curve fitted to Fig. 24b is of the same type as that fitted to Fig. 19 for the rate of development of the early embryos. In this case a more satisfactory fit has been obtained by taking a biological zero temperature of 13 C and a value of K = 58·1. This may be compared with the estimated temperature of no growth, calculated from the regression of each of the 7 series of growth experiments, which varied between 11·4 and 15·9° with a mean value of

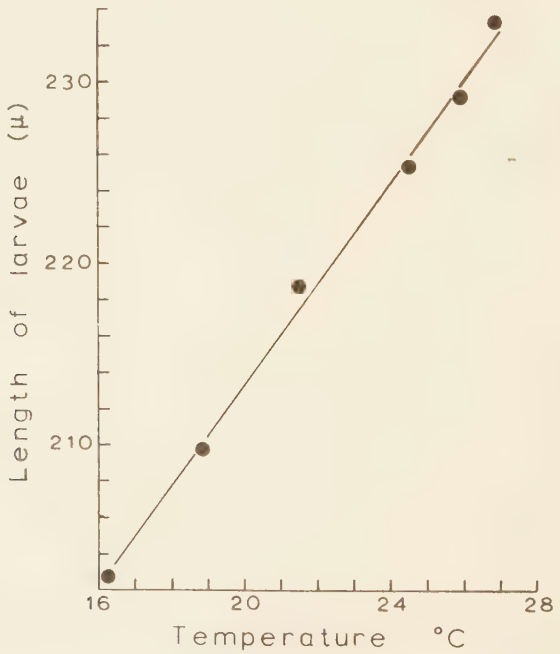

Fig. 22. *The average growth of a brood of larvae at various temperatures over* 48 *hours when fed with* Isochrysis galbana. *Initial mean length* 191·4 μ.

13·8°C. This value for the biological zero of larval growth is about 3–4°C higher than that obtained with the development of the early larvae and also higher than that occurring in the adults (Walne, 1958b). The Q_{10} values over several 2°C temperature intervals (Table 10, column b) show that the Q_{10} of the pelagic larva is higher in the range 17–25°C compared with that of the early embryos. For example, in the range 21–23°C the early embryos have a Q_{10} of 2·2 while the pelagic larvae have one of 4·9 for growth.

In a further experiment the influence of temperature on growth, feeding and assimilation was determined at the same time and on the same brood, and the inter-relationship of these factors explored. A culture of radioactive *I. galbana* was diluted with seawater to give a cell density of 132·4 cells per μl and 100 ml of this dilution added to each of twelve 150 ml glass flasks. 1 ml of a suspension of oyster larvae (mean size 219·6 μ) was added to each flask, giving three to four larvae per ml. Two of the flasks were then placed in each of six water baths, the mean temperatures of which covered the range 15·6° to 25·7°C. The variation in any one water bath during the experiment was not more than 0·5°C. The contents of the flasks were stirred continuously by a stream of air bubbles from a glass jet held near the bottom.

After about 24 hours the cell density in each flask was determined by making eight 0·3 mm³ counts using a haemacytometer. After this the larvae were removed, washed and a random sample of 50 measured. The larvae from each flask were then distributed between two sample trays and assayed for radioactivity. Finally the larvae on the trays were counted under a binocular microscope.

Since estimating the cell density took some time, experiments lasted for 21·25 to 27·25 hours. To bring all the feeding rates into line use was made of the fact that in such an experiment, when cell numbers are plotted on a log scale against time, a straight line results. Such a plot was made for each flask, and the cell densities at 24·00 hours, and at the time the larvae were killed (for comparison with the assimilation figures) were read off. One experiment (at 24·9°C) was found to be defective in that cell consumption, assimilation, and growth were well below expectation and this experiment was rejected. For 11 experiments therefore the cell consumption, assimilation, and growth were determined.

The number of cells of *I. galbana* caught per larva before killing varied from 10,600 to 28,700 and there was a linear relationship between the number caught and the counts per

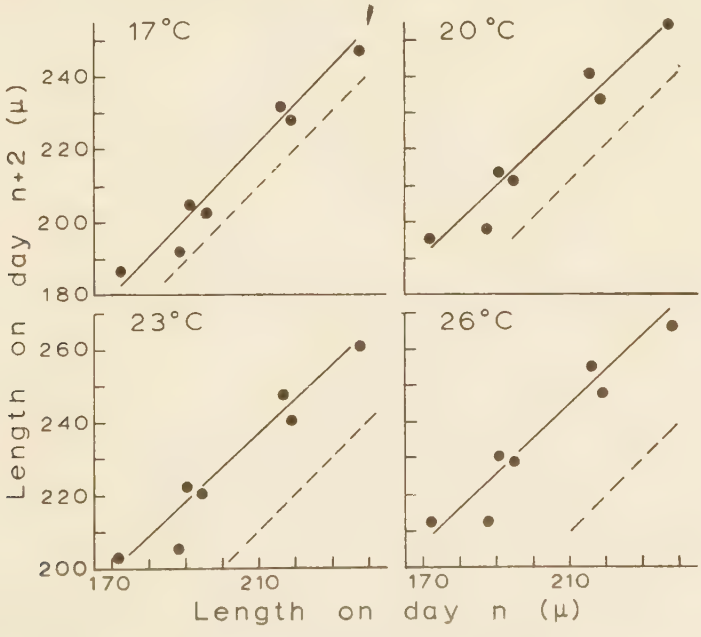

Fig. 23. *The growth in* 48 *hours by larvae at* 17°, 20°, 23°, *and* 26°C. *Calculated from* 7 *series of experiments of the type illustrated in Fig.* 22. *The pecked line is the line of no growth.*

minute per larva resulting from the radioactive assay (Fig. 10d). Throughout the range 10 c.p.m. per larva is equivalent to eating about 3,000 cells. This, therefore, indicates that the same proportion of cells caught was assimilated throughout the temperature range investigated, although at the higher temperatures about three times as many cells were eaten as at the lower temperatures. At the beginning of the experiment 10 c.p.m. was equivalent to 1,490 *I. galbana* cells which suggests an assimilatory efficiency of about 50%. The unchanged proportion assimilated over a three-fold range of consumption is similar to the result obtained when the consumption is varied by different densities of food in the water. Apparently larvae generally assimilate a fixed proportion of the food caught.

Growth in length, which varied between 7 and 20 μ, was very good, and closely related to the number of cells caught (Fig. 25). This experiment demonstrates how the number of cells caught, assimilation and growth are closely related when the feeding behaviour of the larvae is influenced by variation in temperature. The same close relationship has already been shown when feeding is influenced by variations in the concentration of the food.

An example has been given (p. 29) of larvae growing and metamorphosing at the low temperature of 14–16°C and this suggests that very slow growth brought about by low temperature is not harmful to the larvae. Spärck (1929) has demonstrated that oyster larvae are not killed at 1–2°C, but he did not succeed in growing the larvae on subsequently, and his experiment has been repeated. 2,000 recently liberated larvae (mean size 186·2 μ) were

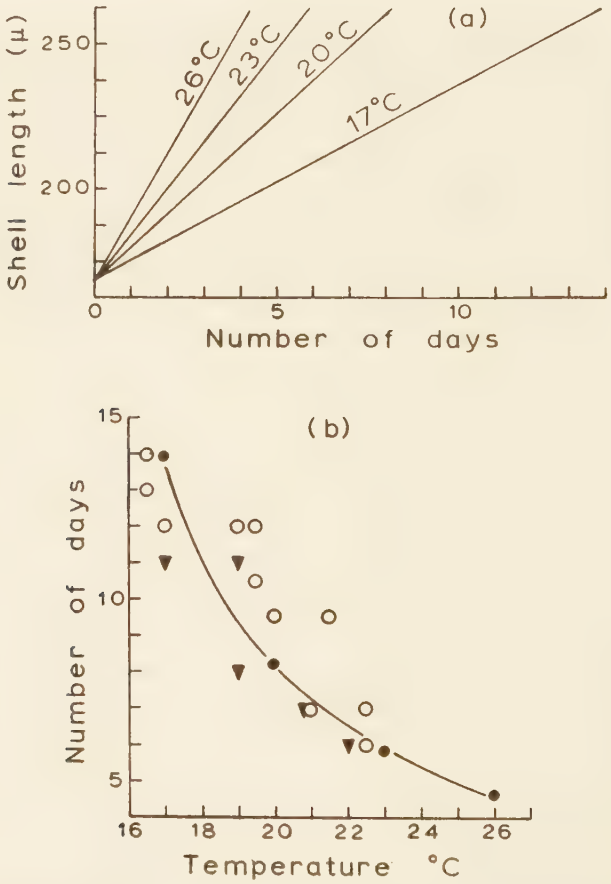

Fig. 24. (a) *Growth curves of larvae starting at* 175 μ *(various temperatures).*
(b) *The time taken for larvae to grow from* 175 μ *to* 250 μ *at temperatures from* 17 *to* 26°C. *Also plotted are the estimated times from liberation to spatfall in the field from Korringa,* 1941 *(open circles) and Waugh,* 1957 *(triangles).*

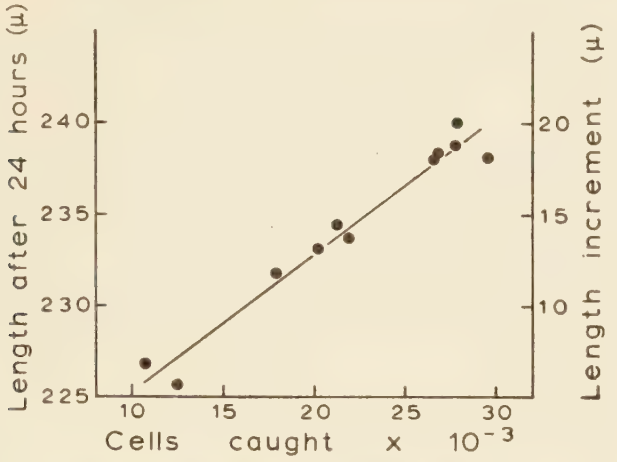

Fig. 25. *The relation between the number of cells caught and growth of larvae in a series of experiments, where feeding was influenced by culturing at temperatures between* 15·6° *and* 25·7°*C. Initial mean length of larvae* 219·6 μ.

put in 1 litre of seawater and the vessel placed in a refrigerator for 72 hours; during this time the water temperature varied between 2·8° and 4·0°C. The remaining larvae of the brood were cultured at 21–23°C. On removal from the refrigerator the larvae looked very thin but the velar cilia were moving slowly and particles were rotating in the gut. The vessel was stood in a water bath at 21°C and *I. galbana* added to the culture. Seven days later a random sample of live and dead larvae was measured and the size distribution of the population (Fig. 26b) shows clearly that about half had grown at about the normal rate while the other half had failed to grow. Many of these were dead. From the results presented in this section it seems clear that the usual variations in temperature occurring during the breeding season are not in themselves harmful to the larvae, and the harm or good comes indirectly from the resulting variations in the growth rate.

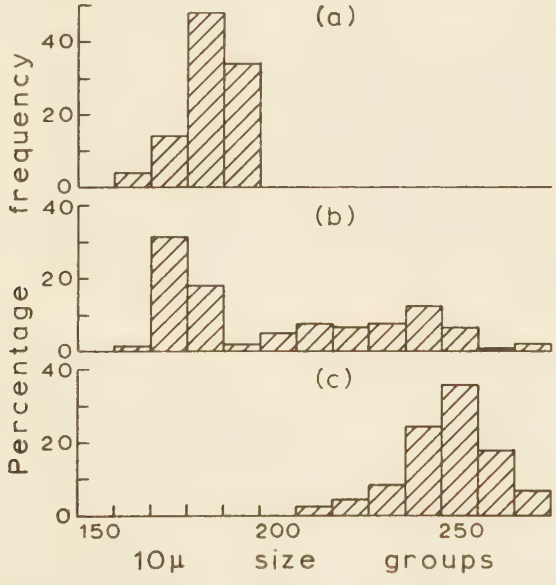

Fig. 26. *The effect of a period of low temperature on larvae.*
(a) *Length frequency at the time of liberation.*
(b) *Length frequency after the larvae had been kept at* 2·8–4·0°*C for* 72 *hours and then cultured at* 21°*C for* 7 *days.*
(c) *Length frequency of larvae which had been cultured at* 21–23°*C for* 6 *days immediately after liberation.*

THE INTERACTION OF VARYING CELL DENSITY AND TEMPERATURE

In one experiment the assimilation of ^{32}P from *I. galbana* was measured at a range of 6 cell densities (25–300/μl) and at two temperatures, 20° and 24°C. The two curves (Fig. 27) show that at all the cell densities tested the uptake at 20° is about 70% of that at the same cell density at 24°C. This is the same ratio as that obtained in feeding experiments at 20° and 24°C with abundant food (Fig. 21), and it demonstrates that temperature has a similar influence on assimilation at a wide variety of cell densities. In a further experiment cell densities of 100 and 10 cells per μl were tested at temperatures in the range 16·4–27·9°C.

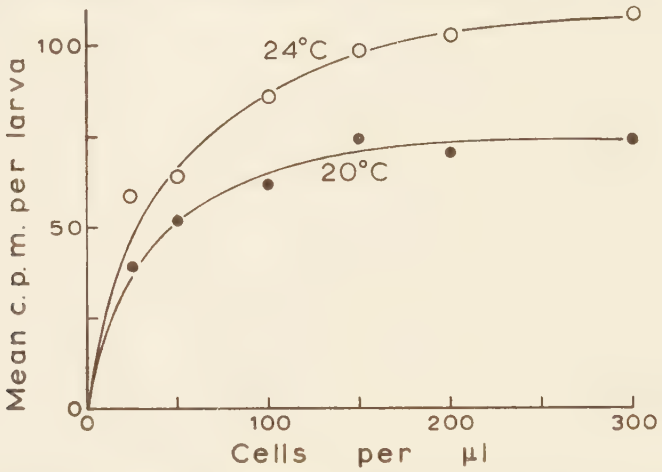

Fig. 27. *The assimilation of* ^{32}P *from* Isochrysis *by a brood of larvae when cultured at various cell densities at* 20°C *and* 24°C.

The results (Fig. 28) show that throughout this temperature range the uptake at 10 cells per μl was constantly about 25% of that at 100 cells per μl.

These two experiments together indicate that the results of feeding at various cell densities and temperatures given in Figs. 6 and 21 can be taken together to calculate the assimilation at any combination of food and temperature.

In three further series of experiments the interaction of temperature and cell density on the growth of the larvae was studied. In each series of experiments larvae were cultured at cell densities of 10, 25 and 50 cells per μl at each of four temperatures in the range of 16–25°C. Because the growth of larvae is not immediately affected when they are transferred from

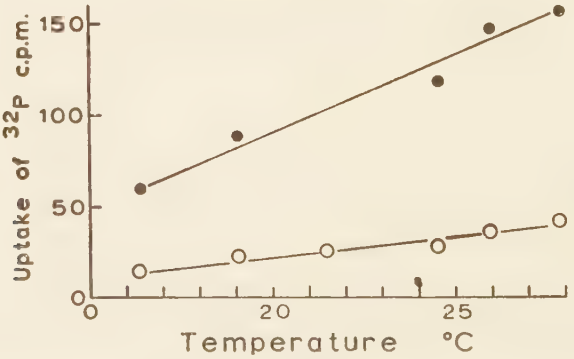

Fig. 28. *The uptake of* ^{32}P *from* Isochrysis *by larvae at various temperatures when fed at* 10 *cells per* μl (*open circles*) *and* 100 *cells per* μl (*solid circles*).

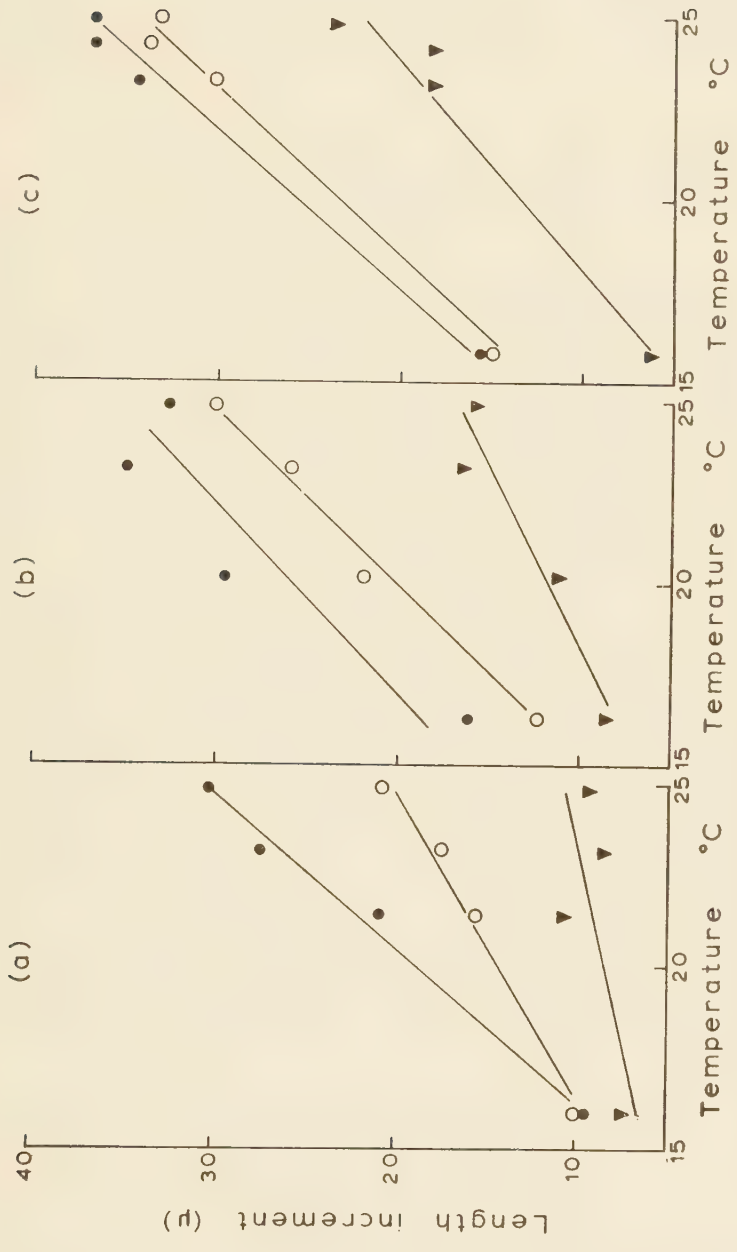

Fig. 29. *The growth of oyster larvae in* 48 *hours related to temperature when fed at* 10 *cells per* μl *(triangles),* 25 *cells per* μl *(open circles) and* 50 *cells per* μl *(solid circles). The initial lengths of the larvae were:* (a) 191 μ, (b) 203 μ *and* (c) 184 μ.

abundant food to reduced food, they were first cultured for 24 hours in the cell density to which they were transferred when the experiment began. The experiments were made in 1 litre beakers in the same manner as the growth experiments with 100 cells of *Isochrysis* per μl. At the end of 48 hours the larvae were killed and a representative sample of 100–200 measured. Samples of larvae from each of the three cell concentrations were measured at the beginning of the experiment.

The data are presented in Fig. 29, a, b and c. For each cell density in each series there is a straight line relationship between the mean length of the population after 48 hours and the temperature.

TOTAL FOOD REQUIREMENTS OF THE LARVAE

THE data presented in this paper give information on the amount of food eaten and subsequently assimilated by the larvae and also on their growth in the presence of an abundance of food at various temperatures. In this section an attempt is made to relate the food requirements of the larvae for energy and growth with the observed assimilation. Such energy budgets can be made at the fundamental level in terms of calories as Richman (1958) has done for *Daphnia,* or by considering the balance in terms of either carbon or dry organic matter. In this paper the budget will be considered in terms of dry organic matter, since in most cases neither the carbon content nor the calorific value is known and only approximate values could be assigned on the basis of similar observations published for other species.

The energy budget can be expressed by the equation

$$\text{assimilation} = \text{growth} + \text{respiration}$$

(Lindeman, 1942, Richman, 1958), where assimilation is the food eaten less that egested. This equation can be solved approximately for oyster larvae.

The relation between assimilation, as determined by the ^{32}P technique, and food density in the water and temperature is known from Figs. 1 and 21, and this relation can be used to calculate an index of food assimilation for various conditions of temperature and food abundance. This is set out in Table 11 for food densities of 10, 25, 50 and 100 cells of *Isochrysis* per μl and temperatures of 17, 20, 23, 25 and 26°C. As Fig. 10 indicates that the proportion of cells caught which is assimilated by the larvae is unaffected by changes in temperature or abundance of food, the table can be used as an index of the number of cells caught by a given batch of larvae under different conditions. For example, if a given population caught 38 x cells at 17°C and 25 cells per μl, then they would catch 120 x cells at 25°C and 50 cells per μl. The value of x depends on the size and vigour of the larvae. Since growth is closely related to the number of cells assimilated, this table can be used to give indices of the amount of growth made by larvae cultured under various conditions of temperature and food. A demonstration of this is given by taking the experiment (Fig. 29b) where

Table 11: *Index of assimilation. Calculated from the ^{32}P experiments and taking* 20°C *and* 100 *cells per* μl *as* 100

Food cells per μl	*Temperature* (°C)				
	17	20	23	25	26
10	19	27	35	40	43
25	38	55	70	82	87
50	56	80	108	120	126
100	70	100	128	150	158

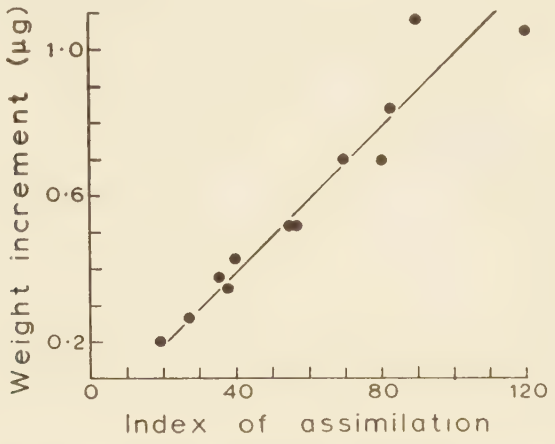

Fig. 30. *The relation between calculated increase in dry weight in* 48 *hours in the experiment reported in Fig.* 29(b) *and the appropriate index of assimilation.*

larvae were cultured at several temperatures and at 10, 25 and 50 cells per µl. The length increments at the standard temperatures of 17, 20, 23 and 25°C have been read off the graph and converted to dry weight increments. These increments and the appropriate indices of assimilation are related in Fig. 30. This clearly demonstrates that the index of assimilation, whether varied by temperature or food supply, is closely related to growth.

The calculated minimum number of cells assimilated by larvae of various sizes at 21–23°C when fed with abundant food is given by the regression in Fig. 15, but it is known from observations on the number of cells removed from the water that larvae can catch two or three times as many cells as the ^{32}P figures suggest are assimilated. Differential or incomplete assimilation may take place or only a proportion of the cells may be swallowed.

An example of the relation between the number of cells assimilated by larvae of different sizes and at different temperatures and the growth increment is given in Fig. 31. The growth increments have been calculated from the average growth curves in the presence of abundant food given in Fig. 24a for temperatures of 17, 20, 23 and 26°C. From these curves the dry weight increment in 24 hours for larvae with initial sizes of 180, 200, 220 and 240 microns has been calculated (Table 12). The minimum number of cells assimilated by these larvae

Table 12: *The total dry weight increment* (µg) *by larvae of various lengths and cultured at different temperatures. Calculated from Fig.* 24a

Temperature (°C)	*Initial length of larvae* (µ)			
	180	200	220	240
17	0·11	0·14	0·18	0·23
20	0·16	0·22	0·27	0·36
23	0·23	0·30	0·37	—
26	0·30	0·42	0·55	—

has been estimated from the regression given in Fig. 15 by assuming that the average temperature of these experiments was 22°C. The effect of different temperatures has been calculated from the relationship between assimilation and temperature given in Fig. 21.

The relationship between cells assimilated and mean weight increment is close (Fig. 31). The number of cells assimilated for a weight increase of 0·1 µg decreases from 4,000 to about 3,000 in the upper part of the range. This may be a true tendency or it may be due to some systematic experimental error. The dry weight increments given are for the whole animal

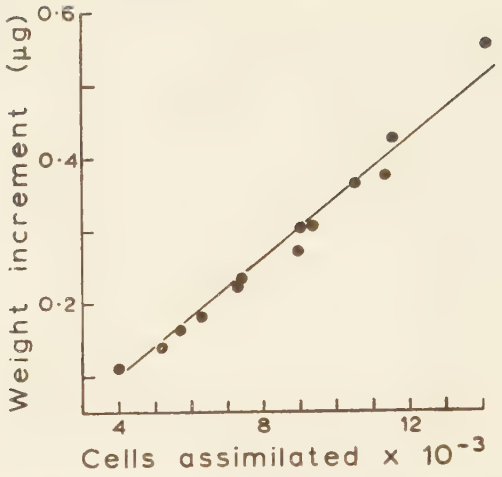

Fig. 31. *The calculated increase in dry weight in* 24 *hours by larvae in experiments reported in Fig.* 24(a), *related to the calculated number of cells assimilated.*

and the increase in dry organic matter is only 25% of these values. In summary, then, an assimilation of about 3,500 cells brings about a dry weight increase of 0·025 μg dry organic matter.

In the *Isochrysis* cultures used in this work, 10^6 cells had a dry weight of about 16 μg (see p. 22). Therefore 3,500 cells weigh 0·056 μg. This is about double the estimated weight increase obtained from this number of cells, but so far no allowance has been made for respiration or excretion during the experiment.

The respiratory rate of oyster larvae has not been measured, but data published by Zeuthen (1947) relating to certain marine bivalve larvae allow an approximate estimate to be made. For *Mytilus edulis, Zirphaea cristata* and *Mya arenaria* larvae of various size and in various states of activity the respiratory rate was of the order of 0·5–1·5 μl $O_2 \times 10^{-3}$/hour/animal at 16°C. In his Fig. 28 the results of a large number of experiments show that in the planktonic stage, the larvae of *Mytilus edulis* respire about 0·01–0·03 μl O_2/hour/μg of total nitrogen. Taking the average total nitrogen content of these small marine animals as 2% of the wet weight, and the dry weight as 20% of the wet weight, the respiration of a *M. edulis* larva of 0·5 μg dry organic matter was 0·5–1·5 μl $O_2 \times 10^{-3}$/hour or 0·012–0·036 μl O_2/24 hours. Assuming that the respiration of 1 mg of dry organic matter requires 1·2 ml of oxygen at normal temperature and pressure, this respiratory rate is equivalent to complete oxidation of 0·01–0·03 μg organic matter per larva per day. An oyster larva of 0·5 μg dry organic matter has a daily increase of 0·12 μg dry weight per day. If its respiratory requirements are of the same order as those of a mussel larva its total daily requirement of organic matter is therefore 0·12 μg for growth and 0·02 μg for respiration, making a total of 0·14 μg. In this case growth accounts for 86% of the food requirement. Even if the respiration per unit weight is double that of *Mytilus*, growth still accounts for 75% of the total food requirement.

A review by Zeuthen (1953) suggests that, in small metazoa, size increase can occur with little reduction in metabolic rate, and this makes it probable that the oxygen requirements of larvae of various sizes are roughly constant on a unit weight basis. As the weight increment on a unit weight basis decreases with increasing size of the larvae, then the proportion of the assimilated food used for respiration will gradually increase.

The calculated daily requirement of 0·14 μg dry organic matter by a larva of 2 μg (229 microns in length, 0·5 μg dry organic matter) is equivalent to 8,750 cells. This may be compared with the estimated 11,500 cells assimilated by larvae of this size (Fig. 15), which indicates that this estimate of the total organic requirement approximately balances with the food assimilated. The estimated 68–80% of the total food assimilated which is utilised for growth may be compared with the estimate of 55–58% made by Richman (1958) with *Daphnia* fed on *Chlamydomonas*. He also quotes other data, for example, *Tubifex* 31·6%, carp 31%, and other fish 10·36%. Jørgensen (1952) made an estimate for *Mytilus edulis*

larvae of 73%, based on Zeuthen's respiratory data and on unpublished data on growth rate in the Baltic, and for two gastropod veligers estimates of 62 and 63%. Gibor (1957) estimated an efficiency of utilisation of 53% for *Artemia*. Harvey (1950) estimated for *Calanus*, using the known growth rate at Plymouth in the summer and its observed respiratory rate in the laboratory, that about 71% of the assimilated food is used in growth and 29% in respiration on the average over the whole life cycle. From this somewhat scanty information it seems probable that of the food assimilated by small planktonic organisms rather less than half is oxidised in respiration, while the remainder is used in growth. Estimates of the food requirements from studies on the respiration will therefore only estimate about half or less of the total requirement.

DISCUSSION

THE experiments outlined in this paper have demonstrated the density at which a food organism, *Isochrysis galbana*, has to be present in the water for maximum feeding and growth of oyster larvae to take place. The influence of temperature on growth and feeding has also been investigated, and it is to be expected that these two factors will exert a dominating influence on the rapidity of growth of larvae in the field. The index of assimilation, detailed in Table 11, has been demonstrated to be closely related to the size increment (Fig. 31). The reciprocal of the index of assimilation (Table 13) is closely related to the time taken to

Table 13: *The reciprocal of the index of assimilation* (*Table* 11) × 100

Food cells per μl	*Temperature* (°C)				
	17	20	23	25	26
10	5·3	3·7	2·8	2·5	2·3
25	2·6	1·8	1·4	1·2	1·1
50	1·9	1·2	0·92	0·83	0·79
100	1·4	1·0	0·78	0·67	0·63

complete any given stage. This is demonstrated by plotting the time taken to develop from 175 to 250 μ, at various temperatures (taken from Fig. 24b) against the reciprocal of the index of assimilation at these temperatures and at 100 cells per μl (Fig. 32). This table

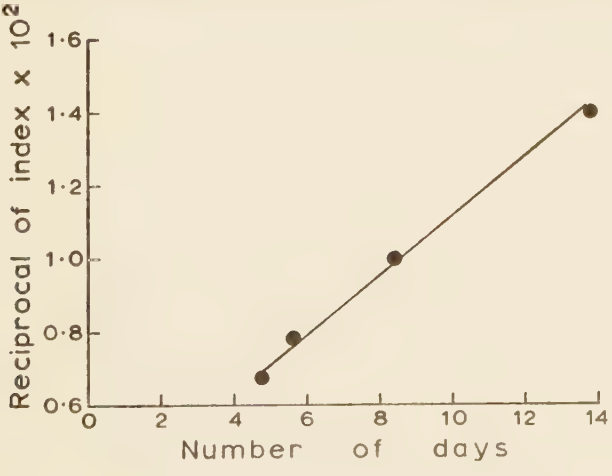

Fig. 32. *The time taken to develop from* 175 *to* 250 μ (*see Fig.* 24(b)) *at various temperatures, and at* 100 *food cells per* μl, *related to the reciprocal of the index of assimilation.*

therefore gives an index from laboratory conditions of the time taken for larvae to complete their pelagic development under various conditions of temperature and food.

Water temperatures in the range 17° to 21° occur commonly over British oyster grounds in the breeding season and small changes in this range make considerable differences in the larval growth rate (see above, p. 32); for example, raising the temperature from 17·0° to 21·5° halves the duration of the pelagic stage. At higher temperatures changes in the rate of development are rather small.

Variation in the amount of food in the water also leads to substantial differences in the growth rate but here comparison with conditions in natural waters is more difficult because of the paucity of field data. The experimental evidence clearly shows that the most rapid growth rates are only obtained with 50 to 100 cells of *I. galbana* per μl, while at 10 cells per μl growth is very slow. In field samples it is usually not possible to identify the species of nannoplankton found, and it is therefore customary amongst workers interested in oyster problems to express the results as numbers of organisms less than 10 μ in size. The longest series of data is from the Essex rivers (Knight-Jones, 1952; Waugh, 1957). Here average densities were of the order of 2–10 cells of 2–10 μ per μl and counts of over 10 per μl were rarely encountered. Cole (personal communication) made a series of observations in 1938 and 1939 in the Helford river where the water is relatively free from silt and very small flagellates can be more readily observed. In both years 20–40 flagellates per μl were frequently observed and up to 100 per μl on occasions, but the majority of these were 2 μ in size or less. Forms 3–10 μ in size were less than 10 per μl. Drinkwaard (1961) in a study of the Oosterschelde in 1959 found 2–4 flagellates per μl, while Millar (1961) working on the west coast of Scotland found only about 1 per μl. In the semi-natural tanks at Conway populations up to 161 per μl have been recorded without enrichment, but more normal levels are about 20–30 per μl (Cole, 1939; Walne, 1956b). Although these data give no information on the species that were being counted, it seems that oyster larvae in the field are often living on densities of flagellates markedly below those which laboratory experiments indicate as optimal. The estimates made on the time taken from liberation to spatfall in the field are not very different from estimates made on laboratory cultures. Therefore food densities in the field must, on occasion, be suitable for near maximal growth.

There are various possible explanations of this discrepancy between field observations and laboratory experiments. It may be that the larvae do not feed so actively in the laboratory and if this is so then there is no discrepancy to resolve, but if the laboratory results are correct, then either the field estimates of nannoplankton density and larval growth rate are incorrect, or the larvae are feeding on something else. It is unlikely that cell densities of 50 to 100 per μl remain undetected by the methods employed, since such densities can be readily counted without prior concentration of the sample. As discussed above (p. 30), it is possible that the speed of development in the field has been over-estimated, but not sufficiently to account for the very low growth rate suggested by the laboratory experiments at 25 cells per μl. A similar discrepancy is found in *Calanus* where the activity in the laboratory is not apparently sufficient for it to maintain itself in the sea (Cushing, 1959). In that case, however, it has not been demonstrated that the laboratory methods of culture are sufficiently good to maintain the *Calanus* in active growth, as has been done for oyster larvae.

Finally there remains the possibility that the larvae are living in part on something other than flagellates of less than 10 μ. Knight-Jones (1952) recorded also the number of diatoms and non-motile forms found in his samples and they were often as numerous as flagellates, but even so his total count of algae only rarely reached 20 cells per μl. A population of 50 and 100 cells per μl of *Isochrysis* represents about 0·8 and 1·6 mg of dry organic matter per litre respectively, and this is well within the estimates of the particulate organic matter in inshore waters of this area (Corner, 1961). There is, therefore, probably sufficient particulate organic matter in the water to agree with the estimates made from the laboratory experiments and it would be worthwhile exploring for other suitable food materials for larvae. Recent experiments by Corner (loc. cit.) suggest that it may be important to consider the particulate organic matter when assessing whether waters contain sufficient food for *Calanus*.

Of what importance is this variation in the length of the larval period? In the field little is known of the causes of mortality in the pelagic stage, but it is generally assumed that the

risks to which larvae are then exposed are greater than those of the subsequent sedentary phase. As many of the risks to which the larvae are exposed (discussed in more detail in the next paragraph) are little affected by fluctuations in the temperature and nannoplankton density, they may be considered to be roughly constant in unit time. The slower the larvae grow, therefore, the greater the mortality.

Larvae may be killed or lost from the general population by the action of currents and storms, or they may be eaten. Losses by the first category are clearly independent of temperature or food supply. The majority of dense populations of oysters are in inshore bays and estuaries and, while very wide dispersion of the larvae may be valuable to the species, the majority of such larvae will be swept away from suitable areas. The amount of water lost from the area of the breeding stock is therefore important. Korringa (1941) estimated for the Oosterschelde that 3·7% of the water (and its contained larvae) was lost each tide into the North Sea and about 65% after 14 days. The numbers lost by turbulence caused by rough weather is unknown, but some must be killed by swirling sand particles, by becoming lodged on the bottom or even by being thrown onto the beach.

Several planktonic predators of oyster larvae have been noted in the literature (Nelson, 1925; Cerruti, 1941), but no quantitative data are available. Mileikovski (1959) estimated from plankton samples in the White Sea that the larvae of *Nepthys ciliata* in two hours consumed 3% of the pelagic larvae of *Macoma baltica* and *Mya arenaria*. In shallow waters, the bottom fauna must be important and some calculations with respect to adult oysters illustrate this point. Oysters are known to eat larvae Savage (1925) recorded up to 112 in the stomach of one oyster from the Orford river, and it is probable that some had been rejected in the pseudofaeces. Although some larvae may pass through the gut alive, the great majority which are entangled in faeces or pseudofaeces, and then thrown on the bottom, must subsequently die. Adult oysters will pump about 10 litres per hour and all particles as large as larvae will be trapped. Many oyster grounds are quite shallow – much of the rivers Crouch and Roach, and the Oosterschelde, is less than 8 m. For the purpose of this example take a mean depth of 10 m and assume sufficient turbulence to keep the water column mixed. An oyster ground can readily support 10 to 100 oysters per sq. m., but if oysters are absent other filter feeders will probably be present to a similar density. At 10 litres per hour the two densities of oysters will sweep free 2,400 and 24,000 litres per day respectively. By substituting the various values given above in the formula for calculating the volume swept free given on p. 10, the loss of larvae, assuming that all that are swept in by the feeding current are lost, can be calculated. It is 21% and 91% per day respectively. In practice the absence of complete turbulence, the habit of the larvae to swim upwards, and washing into deep water at low tide, will reduce these losses.

The data outlined in the above paragraphs indicate that the daily mortality of larvae may reach a considerable figure, perhaps 30 to 40% per day from causes which will vary little with small changes in temperature and food. The survival of fair numbers of larvae will therefore be aided both by rapid growth, so as to reduce the duration of exposure to these risks, and by a large stock of larvae initially in the water, since many of the causes of loss act in a proportionate manner.

SUMMARY

Details of experiments on the influence of variations in quantity of food and temperature on the larvae of *Ostrea edulis*, both in the pelagic phase and before liberation, are given. The techniques used to observe the reaction of the larvae were measurement of growth, the rate of removal of algal cells from the water, and the assimilation of ^{32}P from the food. Using *Isochrysis galbana* as food, it was found that assimilation and growth were closely related to small variations in cell density up to about 50 cells per μl, but above this level substantial increases in the cell density caused only small increases in growth and assimilation. Tests with 6 other algal species demonstrated how larvae become relatively satiated with food at much lower cell densities when the cells were larger than when they were small. Larvae start to feed at a size slightly smaller than that at which they are normally liberated.

Development within the mantle cavity takes about 12 days at 16–17°C, 8 days at 19 20°C and 6 days at 22–23°C. In the pelagic phase the time taken to grow from 175–250 μ decreases from 14 days at 17°C to 5 days at 25°C. Assimilation, growth and cells caught were closely inter-related when the feeding behaviour was influenced by variations in temperature and food supply. Analyses of the food requirements of larvae indicate that about 75–85% of assimilation is required for growth and the remainder for respiration. The ^{32}P data indicates that 13–50% of the food caught is assimilated, but the proportions of the fraction not assimilated which are either rejected before eating or incompletely assimilated are not known.

The importance of a rapid growth rate to larvae in the sea is discussed and attention is drawn to the hazards which kill a proportion of larvae each day.

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